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REPORT OF THE INTERNATIONAL PLAGUE CONFERENCE.

Held at Mukden, April, 1911, under the auspices of the Chinese Government.

Edited by ERICH MARTINI, G. F. PETRIE, ARTHUR STANLEY, AND RICHARD P. STRONG.

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THE PHILIPPINE

JOURNAL OF SCIENCE

B. THE PHILIPPINE JOURNAL OF TROPICAL MEDICINE

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THE RÔLE OF STOMOXYS CALCITRANS IN THE TRANSMISSION OF TRYPANOSOMA EVANSI.[1]

BY MAURICE BRUIN MITZMAIN.

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This paper is one of a series of studies undertaken to incriminate, or to exclude from future practical consideration, the various species of bloodsucking insects that might be concerned in the transmission of surra. It is planned to duplicate these methods of experimentation, if practicable, with each species of ectoparasite of the draft animals of the Philippines. It was believed that this investigation could be made more valuable, and a more practical insight obtained into the problem of the epidemiology of surra, by such an intimate study of each species at close range, than by attempting a more or less hasty survey under field conditions of the several species of flies implicated in natural outbreaks of the disease.

Bagshawe, editor of the Sleeping Sickness Bureau Bulletin, has written in this connection as follows:

> It appears that an epidemic of surra may be started from an animal in which trypanosomes are very scarce. If it were known that one species of insect alone is capable of becoming infected from such an animal and that flies of this species transmit infection to other animals for a period of many weeks, such knowledge could not fail to lead to precision and hence economy in preventive methods.

In most of the literature concerning *Stomoxys* and the transmission of trypanosomiasis, the species of the insect carrier has not been determined. This can be pardoned when one realizes that the work has rarely been performed by entomologists, but

[1] To be published as Bulletin No. 24, Bureau of Agriculture of the Government of the Philippine Islands.

[2] Archibald R. Ward, chief.

mostly by medical men or veterinarians who are more vitally concerned in field operations. The species of fly under investigation and discussed in the present paper has been compared carefully with material brought from California and with the descriptions of the oriental species of *Stomoxys* recently compiled by Summers. (1)

From recent reports, the workers in the field, especially in tropical Africa, have been perplexed to account for the spread of trypanosomiasis in the absence of tsetse flies. Many investigations have followed in efforts to discover other insect carriers. Species of *Stomoxys* have been incriminated in many cases; in other instances the genus *Stomoxys* has been eliminated. Species of *Stomoxys* have been cited in the general literature as carriers of pathogenic trypanosomes by the following writers:

Bruce(2) found in Uganda that swarms of *Stomoxys* might bite infected and healthy animals freely without conveying the infection (nagana).

Bruce and others(3) in concluding a discussion on work done with *Trypanosoma pecorum*, the cause of cattle disease in Uganda, note that the carrier is probably not a *Stomoxys*.

Montgomery and Kinghorn(4) advance the view that the Rhodesian cattle trypanosome can be transmitted by *Stomoxys calcitrans*.

Dutton and Todd(5) failed to infect animals with *Tr. gambiense* or *Tr. dimorphon* by bites of *Stomoxys* which had been either freshly caught from an enzoötic focus or had been fed previously on an infected animal.

Theiler's(6) attempts to transmit m'bori, a nagana-like trypanosomiasis, with *Stomoxys* from horse to horse were negative.

Sander(7) mentions that he has demonstrated in East Africa that nagana of cattle is transmitted by *Stomoxys*.

Martin, Lebœuf, and Roubaud(8) advance the opinion that in experiments with flies "as simple carriers," the species of *Glossina* are of more importance than those of *Stomoxys;* yet the rôle of the latter can not be overlooked."

Novy(9) states that *Stomoxys* is apparently incapable of spreading the infection of sleeping sickness.

Austen(10) records a note by Captain Haslem of the finding, in 1898, of *Tr. brucei* in the abdomens of *Stomoxys* caught sucking the blood of sick mules.

Castellani and Chalmers(11) state that *Stomoxys* is suspected of spreading surra and that *Tr. evansi* appears to develop in the stomach of *Stomoxys*.

Sivori and Lecler(12) succeeded in infecting horses with *Tr. equinum* by subjecting them to bites of *Stomoxys* which had sucked the blood of sick animals.

Sieber and Gonder,(13) working with dourine in Hamburg, came to the conclusion that it was not unlikely that *Stomoxys calcitrans* was the responsible carrier in the infection produced.

Schat(14) concludes that *Stomoxys* is the chief agent in the propagation of surra among cattle and horses in Java.

Manders(15) states that in Mauritius *Stomoxys geniculatus* is almost

certainly the carrier of surra and that the mortality is greatest during the months when this fly is most prevalent.

Daruty[16] says that in Mauritius *Stomoxys nigra* plays the same part in the spread of surra as the tsetse fly does in Africa in the spread of nagana.

Diexonne[17] notes the fact that a surra epizoötic broke out in Mauritius simultaneously with the appearance of *Stomoxys*.

Laveran and Mesnil[18] mention *Stomoxys* as a probable transmitter of surra.

Mohler and Thompson[19] state that in the outbreak of surra among the imported cattle landed on an island near New York, *Stomoxys calcitrans* probably was not a factor in the spread of the infection. A species of *Tabanus* was incriminated.

Darling,[20] in discussing the mode of transmission of *Tr. hippicum*, the agent of murrina, believes that it is extremely unlikely that *Stomoxys calcitrans* conveys the infection in the Panama Canal Zone.

Sander,[?] in discussing surra in the Philippines, asserts that *Stomoxys calcitrans* is proved by Curry in 1902 to be the carrier, and that this was confirmed by Musgrave and Clegg.

Leese,[21] in discussing the comparative practical importance of *Stomoxys* as a surra transmitter, points out that, in an epizoötic, *Stomoxys* does not perform its work until the infection has already been introduced by a more capable carrier like *Tabanus* which usually transports the organisms from a distance. *Stomoxys* transmits under these conditions at close range, and usually among individuals in the same herd.

Curry[22] found the trypanosomes of surra still active in the proboscides and stomachs of *Stomoxys calcitrans* twenty-four hours after they had bitten an infected animal. He believes that *Stomoxys* is alone responsible for the propagation of surra in the Philippines. No experimental evidence is advanced.

Musgrave and Clegg[23] state in their report on surra in the Philippines, that they transferred the infection to the monkey, horse, rat, dog, and guinea pig by biting flies, in experiments so guarded as to make the results absolutely conclusive.

The inconclusiveness of the last reference is shown in the fact that the species of biting fly concerned is not given, nor are data of experiments involving procedure of method in obtaining results submitted. One is left to assume that the direct mechanical method was applied and the biting fly used was *Stomoxys calcitrans*. These omissions are noticeable in contrast to the general excellence in the carefully compiled literature and observations embodied in the remainder of the report.

Bagshawe,[24] in commenting on the consensus of opinion of authorities on surra, writes that it is *generally assumed* that flies act as mechanical transmitters or accidental carriers, but, as far as he is aware, no experiments are on record which prove or disprove it. This is before the appearance of Leese's paper in 1911.

MECHANICAL TRANSMISSION.

That the principal and probably the only method by which *Stomoxys* transmits the trypanosomes of surra and of other

trypanosomiases is purely mechanical has been taken for granted, and, in a few instances, proved by experiments. By the mechanical method it is assumed that there is involved a direct conveyance by the insect carrier of infection either immediately or in varying periods up to forty-eight hours after feeding on the infected host.

Novy, MacNeal, and Torrey,[25] referring to insects such as *Stomoxys*, affirm that the facts, such as they are, indicate that the flies merely transmit the surviving unaltered trypanosomes which have been taken up with the blood, and that such transmission can occur only during the few hours following the infective feed.

Evans[26] cites *Stomoxys* as the mechanical carrier of the trypanosomes of elephant surra. No experimental evidence is offered.

Jowett[27] obtained negative results in mechanical transmission experiments in cattle trypanosomiasis with *Stomoxys* fed on rats, sheep, and goats.

Fraser and Symonds,[28] working with surra and the mechanical transmission method with *Stomoxys*, were unsuccessful in five experiments. In two instances 8 and 11 flies, respectively, were used.

Gaiger[29] states that in some parts of India *Stomoxys* is found where there is an absence of Tabanidæ and the former is probably equally capable of transmitting surra mechanically.

In my experiments covering this point, an attempt has been made to view the problem exhaustively from every possible angle. First, the flies, transferred from their infected hosts, have been permitted to *complete* the initial feeding prior to biting the healthy animal. Second, the flies, feeding on the surra host, have been *interrupted* within a minute, after which they were transferred to a healthy animal to complete their meal. Third, this *interrupted* feeding was repeated during several hours of the day and through a period of several days. In these tests, guinea pigs, monkeys, and horses have been employed. It was found that the species of susceptible mammal used made no difference in the results of the experiments. The use of animals other than the natural hosts of *Stomoxys* has been criticized by several workers. A limited experience convinces me, so far as many negative results can convince, that the reaction to biting of infected flies is similar in all the hosts cited.

The experiments were carried on with animals in a large cage, or in a glass jar, or in the open with the host immobilized. In the first case the insects were placed in the cage or in the jar and permitted to bite at will. By the second method they were applied in a large bottle to the tail of the animal, and also individually in test tubes. Both wild flies and laboratory-bred flies were used, and in each case this condition is stated.

In all of the experiments reported upon in this paper the

failure to infect was concluded only after the following examinations. The temperatures of the horses and monkeys were registered twice daily. Microscopic examinations of the blood of horses, monkeys, and guinea pigs were made each day. In these examinations never less than 30 fields per blood film were scanned. In the experiments with horses and monkeys an additional control was provided by guinea-pig inoculations. From the horses 2 to 6 cubic centimeters of blood were drawn and inoculated, from the monkeys 2 to 3 cubic centimeters. One guinea pig was used for each animal tested.

The animals were held in quarantine, both before and after each experiment, in fly-screened sheds or cages; the horses in fly-screened enclosures provided with a suitable double-door vestibule, and the monkeys and guinea pigs in individual cages which were made fly proof with double screens of close-meshed gauze.

Unless otherwise stated, in every experiment included in this paper, an animal exposed to flies, whether through biting or subcutaneous inoculation, was examined daily for thirty days and afterward never less than twice weekly for ten days.

EXPERIMENTS WITH HORSES IN A SCREENED STABLE.

The experiments with the horses and *Stomoxys* were conducted in a specially constructed fly-proof stable made to simulate natural conditions as nearly as possible. The enclosure (Plate I) utilized in the present series was of the following description.

The walls and top were screened with brass wire of 16 to 18 meshes per inch. No roof was provided, and the animals were protected from the sun by canvas screens stretched overhead. A section was devoted to the growth of foliage provided by acacia trees and hedge plants. A constant flow of water was provided to aid in cooling the air within the enclosure. The manure was not removed from these quarters, but was permitted to accumulate in ant-proof wooden tubs at a little distance behind the horses. The animals were watered from removable iron containers within the mangers, filled by individual faucets. The screened enclosure was divided into two parts by means of a low partition formed of half-inch-mesh iron wire, ample for the ready interchange of flies, and high enough barely to prevent contact of the animals. A frame door closed the opening from one stall to another, and a capacious fly-proof double-doored vestibule served as a general entrance into the cage.

There were employed 26,666 flies collected from animals known to be surra-free, but the possibility of the migration of flies from infected sources is not ignored. These flies were placed, after they were etherized and examined, in the fly shed with horse 49, the blood of which contained many trypanosomes, every day during the six days of fly infestation.

Horse 49 which was used to supply infection contracted surra through subcutaneous inoculation March 8, 1912, of the blood of bull 3148, a carabao strain of infection. The blood inoculated from the bull into the horse contained an average of 8 trypanosomes to 30 fields. The incubation period of the disease in horse 49 was six days when, on March 14, 10 trypanosomes per field were seen in a drop of blood. The temperature rose from 37°.8 C. on the morning of March 14 to 40°.2 C. on the evening of that day. The following morning the temperature registered 39°.3 C., when blood was drawn to inoculate monkey A. This animal showed a positive reaction by a rise of temperature of 2 degrees above normal and the presence of trypanosomes in its blood upon the fourth day after injection of the blood of horse 49. The monkey died nine days later, showing marked emaciation and also numerous flagellates in its heart's blood.

During the six days of fly infestation, horse 49 did not show any marked symptoms of the disease, but two days later, March 22, there was a catarrhal discharge from the nose and a congestion of the conjunctivæ. Œdematous swellings manifested themselves beginning April 1, when the breast and forelegs were involved. This condition became more marked daily, until near the time of death when the œdema extended along the abdomen and involved the genitalia. The blood of horse 49 showed numerous trypanosomes daily from March 15 to March 20, after which time the animal was examined daily until April 12 and thrice weekly from that time to the time of death, which occurred May 7, 1912. The heart's blood was then swarming with trypanosomes, and the lesions found were characteristic of surra.

In the present experiment the flies were placed in the cage on the dates mentioned in the following numbers:

Date.	Number of flies.
March 15	3,000
March 16	5,000
March 17	6,874
March 18	3,000
March 19	6,804
March 20	1,988
Total	26,666

Careful note was taken at least five times daily of the approximate number of flies biting the diseased animal, horse 49. At no time during the six days were there fewer than 100 flies biting the horse. On the morning of March 20 there were estimated to be 350 flies either biting or resting on the horse. The horse was in the early stages of the infection from the seventh day to the thirteenth day, and capable of actively resisting the attacks of the flies. That the flies did draw blood from the infected host was demonstrated by microscopical examination of the emulsified abdominal contents of several of them at various times during the course of the experiment. Trypanosomes in moderate numbers were found in fresh preparations and in stained smears.

Horse H–4.—This healthy horse was the first used for exposure to the flies possibly contaminated with surra blood. The infected horse was first removed after a bath with cresol to drive off its parasites, and the stall was similarly sprayed thoroughly with the disinfectant. No. H–4 was not placed in the stall occupied by No. 49, but in the adjoining stall, where by this time the flies had been driven. No. H–4 was exposed for twenty-four hours, during which time it was observed at least five times to be infested by flies to the extent of 10 to 100 flies.

Horse 269.—Six hours after the removal of No. 49 it was replaced by the second contact, No. 269. The fly infestation had noticeably decreased both within the cage and on the horses. Before the end of twenty-four hours the number of flies had been reduced to a few thousands and the horses received the bites of relatively few, probably not more than 40 per cent, at the close of the period of experimentation.

Horse H–3.—The two horses in the foregoing experiments were withdrawn, after carefully ridding them of the flies they harbored, and were superseded by No. H–3 which served as host from the twenty-fourth to the forty-eighth hour. The fly infestation was marked by few bites, never more than 15 at any one time of the five daily observations.

The cause of the decrease in the number of flies was ascertained to be the depredations of four or five voracious lizards which were the survivors of hundreds killed by systematic spraying prior to the introduction of the flies. These lizards were observed to invade the ceiling and walls of the cage, and with characteristic darting movements destroy an astonishing

number of resting flies. One lizard consumed in three minutes 68 flies by actual count.

This condition, needless to say, rendered unfeasible any scheme of experiment except that involving direct mechanical transmission. Therefore, this course of experimentation was not pursued beyond a forty-eight-hour period.

Horses H–4, 269, and H–3 have showed no evidence of infection. The usual tests for the presence of trypanosomes were applied, such as registration of temperatures, blood examinations, and guinea-pig inoculations, and all with negative results. The blood of the horses was examined daily for thirty days. Their temperatures were taken during a course of forty-three days. No reaction was noted. Two cubic centimeters of blood from each of the 3 horses were inoculated into 2 guinea pigs on April 1, at which time no organisms were seen by microscopic examination. The 6 guinea pigs were examined for trypanosomes daily for a period of thirty-five days. They were negative upon every occasion. Horse 269 died June 13, 1912, from an unrecognized cause believed not to be surra. Horses H–4 and H–3 were alive and free from surra on August 15, 1912.

EXPERIMENTS WITH GUINEA PIGS IN A GLASS JAR.

In the following experiments closer observation was made possible by the use of the largest museum jars obtainable, which were screened with close-meshed surgical gauze. (Plate II.)

The method employed was to immobilize a closely cropped surra guinea pig on a wire frame, place it in the jar with a known number of laboratory-bred *Stomoxys*, and replace it at stated intervals, after the flies had fed voluntarily, by a healthy animal similarly prepared. The exchange was made by the aid of ether, the jar being lightly etherized so that flies near the opening would be driven back, and those feeding upon the animal stupefied. The new animal was prepared during the interim and substituted while the flies in the jar were still under the effects of the ether. In the substitution of one host for another the flies recovered from the anæsthetic within a few minutes, in each instance by the time the new animal was introduced and the jar screened. In the case of guinea pig 81, 6 flies commenced feeding on the new host in less than five minutes after the surra guinea pig was withdrawn from the jar.

The experiments were conducted during the daytime, beginning as early as practicable and ending before twilight. The

hosts and parasites were thus under ready observation and very accurate notes could be obtained. The number of flies biting was noted, and the guinea pigs were left in the cage until the flies were apparently satisfied.

On the fourth hour of the experiments the presence of the trypanosomes in the feeding flies was demonstrated in several flies taken from the jar. An emulsion of the stomach and intestinal contents showed abundant trypanosomes. These were present on an average of 15 parasites per field, nearly as many as found in the blood of the original host, guinea pig 35.

The surra guinea pig 35 received its infection through inoculation of an emulsion of a single specimen of *Stomoxys*. This fly had been fed five minutes on a surra guinea pig and six hours later was emulsified and examined microscopically. Numerous surra-like organisms were present prior to inoculation into guinea pig 35. This animal was first positive microscopically five days following injection, and died of surra on the forty-second day.

The experiment was begun on April 2 with 300 newly emerged *Stomoxys calcitrans* and heavily infected guinea pig 35. The following table represents the data compiled:

TABLE I.—*Flies fed on guinea pigs in jar.*

Guinea pig used.	Time after feeding on infected animal.	Number of flies counted on animal.	Length of time fed.
			Hours.
Surra infected: No. 35		225	3
Healthy animal:			
No. 81	5 minutes to 6 hours	155	6
No. 84	20 hours to 25 hours	90	5
No. 87	46 hours to 53 hours	70	7

The three healthy guinea pigs used in this experiment were held in quarantine for a period of forty-five days. They had been examined by the microscope twice to thrice weekly, but in no case were trypanosomes discovered. It was not thought necessary to waste animals in proving that the guinea pigs used were susceptible by blood inoculation. Subsequent experiments give sufficient evidence on this point.

Therefore, it is concluded that under the conditions stated *Stomoxys* did not convey surra by direct mechanical infection.

moment they were used to supply the infected blood. The number of trypanosomes per field is not given in the table.

Guinea pig 45, which had been infected by an inoculation of blood from carabao 3252, underwent an incubation period of five days, and died of surra twenty-six days after inoculation.

Monkey R was inoculated with blood from monkey A, drawn while the latter was dying of the disease.

The infection in monkey A was very light, trypanosomes in the blood being scanty. Monkey R reacted with the presence of surra organisms within four days, showing the usual febrile changes, followed in a few days by other characteristic symptoms. This monkey lived for twenty-two days after inoculation, showing a moderate number of trypanosomes in blood from its heart. The lesions showed characteristic changes of surra.

Monkey F was inoculated with a carabao strain of surra from bull 3148. Seven days after inoculation trypanosomes were recovered from the monkey's blood. It died fifteen days after inoculation, showing all the appearances of trypanosomiasis with characteristic lesions and a moderate number of flagellates in the heart's blood.

Monkey A, as has been previously noted, reacted to an injection of the blood of horse 49. In the present experiment it was used shortly before the death at a time when the blood showed only a moderate number of trypanosomes. The blood was used, while the animal was dying, to infect monkey R, which reacted with a heavy infection.

TABLE II.—*Mechanical transmission by Stomoxys on guinea pigs and monkeys, after completed feeding.*

No. of experiment.	Infected animal used.	Healthy animal used.	Number of flies fed on first host.	Length of time fed on infected host.	Interval between feedings.	Flies feeding at time of transfer.	Number of flies fed on second host.	Length of time fed on second host.
				Hrs. mins.	*Hrs. mins.*			*Minutes.*
1	Guinea pig 45	Guinea pig 18	2	0 11	0 20	None	2	12
2	do	Guinea pig 53	11	1 32	6 0	None	11	41
3	Monkey R	Monkey 3M	150	1 0	24 0	None	43	75
4	do	Monkey 2M	12	0 20	0 10	None	12	30
5	do	Monkey 2L	29	0 35	0 12	None	28	20
6	do	Monkey 3L	12	0 30	0 20	None	7	25
7	Monkey F	Monkey 2G	25	0 28	0 18	None	10	40
8	Monkey A	Monkey C	300	2 0	20 0	None	182	50

In each instance, after a lapse of six weeks to two months, the experiments yielded negative results. The healthy monkeys and guinea pigs used in these experiments have since been employed for other experiments.

AN ATTEMPT TO DEMONSTRATE WHETHER OR NOT A SMALL NUMBER OF FLIES ARE CAPABLE OF TRANSMITTING THE DISEASE.

Tables III and IV represent a single feeding of 1 to 3 flies in an effort to determine the minimum number of flies required to convey the organisms of surra. In the first series 17 experiments were made with wild flies. The flies were fed on surra guinea pig 20, and were not fed again until the time noted, a range of twenty minutes to three days.

Guinea pig 20 was used as the blood donor three days prior to its death, at which time the blood swarmed with trypanosomes. This animal reacted to an inoculation of the blood of a mule dying from trypanosomiasis. The blood was moderately supplied with trypanosomes, and at death the latter animal showed prominent lesions of the disease. Guinea pig 20 was not examined until death, on the fifty-first day after inoculation. The organs showed the general appearance of surra lesions, and the heart's blood fairly swarmed with trypanosomes.

In the second series of experiments a single laboratory-bred fly was permitted to feed once daily on a new guinea pig. The primary bite on the infected animal was of only three minutes' duration. The fly was applied to a guinea pig heavily infected with surra. This animal, guinea pig 35, was in the first stages of the disease, although trypanosomes were abundant in its blood. It was used previously in experiments with flies placed in a museum jar.

In this test thirty-one animals were used, each being bitten once by the fly which fed until apparently satisfied. The feeding with this one fly consumed thirty-one days, and the experimental animals were held for examination for a period of at least forty-two days prior to being declared negative. Blood examinations were made daily for thirty days, after which the examinations were discontinued until the day the animal was employed for a new experiment. Then the examinations were resumed.

TABLE III.—*Series of transfers of several Stomoxys.*

Interval after feeding on infected animal.	Number of flies used.	No. of healthy guinea pig used.	Length of time fed on new host.
Days, hrs, mins.			*Mins. secs.*
20	2	18	12 00
20	1	34	3 00
30	1	10	2 00
1 30	2	6	3 00
15 00	1	8	1 00
21 00	1	27	3 00
1 00 00	2	13	10 00
1 00 00	2	25	6 00
25 40	1	51	40
27 00	1	35	4 00
39 00	1	23	1 00
44 00	1	31	2 00
2 00 00	1	11	4 00
2 00 00	2	15	18 00
64 00	1	7	4 00
64 00	1	38	40 00
3 00 00	3	17	14 00

TABLE IV.—*Series of transfers of a single Stomoxys.*

Interval after feeding on infected animal.	No. of healthy guinea pig used.	Length of time fed on new host.	Interval after feeding on infected animal.	No. of healthy guinea pig used.	Length of time fed on infected animal.
Days. hrs.		*Mins. secs.*	*Days.*		*Minutes.*
21	39	2 00	21	68	6
2 00	22	30	22	71	4
3 00	32	1 00	23	73	4
4 00	37	3 00	24	75	6
6 00	12	6 00	25	77	5
8 00	42	6 00	26	79	3
9 00	16	5 00	27	81	4
10 00	28	3 00	28	83	4
11 00	1	5 00	29	85	4
12 00	52	6 00	30	87	5
13 00	54	2 00	31	90	6
14 00	57	9 00			
15 00	59	6 00			
16 00	61	6 00			
17 00	63	4 00			
18 00	40	5 00			
19 00	65	4 00			
20 00	66	3 00			

The experiments of both series were concluded with negative results.

Flies bred in the laboratory were used in this series, and they were discarded after each experiment. The interval of time between feeding on an infected animal and a healthy animal is stated in the table as the approximate average per fly. The flies were not fed individually, but from a common bottle into which the monkey's tail was introduced. (Plate III.)

TABLE V.—*Mechanical transmission by interrupted feeding of Stomoxys on monkeys.*

No. of experiment.	Infected monkey used.	Number of trypanosomes found.	Healthy monkey used.	Number of flies used on infected monkey.	Length of time flies were applied on infected monkey.	Interval between feedings, approximate average per fly.	Flies feeding at time of transfer.	Number of flies fed on healthy monkey.	Length of time fed on healthy monkey.
					Minutes.	*Minutes.*			*Minutes.*
1	F	Scanty	D	9	18	2	4	9	20
2	R	Moderate	C	15	4	1	15	15	15
3	R	Numerous	S	22	15	3	11	22	20
4	F	Moderate	M	5	2	2	4	15	18
5	F	Numerous	P	19	53	2½	7	19	60
6	F	do	B	5	5	2	5	5	20
7	F	Moderate	L	9	20	3	2	9	24
8	F	Numerous	H	12	6	2	6	4	18
9	F	Swarming	J	12	20	2	5	12	30
10	F	Numerous	N	2	3	2	2	2	7
11	F	do	2C	9	18	2	4	9	20
12	F	Swarming	2O	9	12	2½	7	9	60
13	F	do	2S	18	35	3	15	18	60
14	F	do	2M	1	2	3	1	1	3

The negative results of this series are checked in one experiment by the inoculation of surra blood from an infected bullock into the tail of monkey B. The animal reacted first on June 10, 1912, and died on June 22, 1912.

The relatively long interval between feedings is accounted for by the fact that much time was consumed in manipulating the tails of the respective monkeys, in forcibly interrupting the biting of the flies, and the renewed processes on the second host.

In Table VI, 11 experiments with an equal number of guinea pigs are represented. Here the flies were more easily controlled in the element of time, and, for purposes of close observation, each fly was fed from an individual test tube. The time was accurately noted with respect to three considerations; namely, feeding on the surra host, the interval interrupting the feeding during the transfer, and the completion of the meal on the healthy

animal. The time elements of these are averaged per fly in each experiment.

The surra guinea pig 127 used to supply the infection in these experiments was infected through subcutaneous inoculation of an emulsion of house flies which showed a great number of trypanosomes as the result of feeding on the abraded tail of a surra monkey. This monkey died showing marked lesions of the disease at necropsy. Guinea pig 127 was used when its blood was positive for trypanosomes, for a great number of experiments outlined in this paper. The animal died on the sixty-fifth day of the disease, at which time blood from the heart showed an exceedingly rich infection.

TABLE VI.—*Mechanical transmission with interrupted feeding of Stomoxys on guinea pigs.*

No. of experiment.	Surra guinea pig used.	Condition of blood of donor relative to trypanosomes.	No. of healthy guinea pig used.	Number of flies applied.	Length of time fed on surra host (average per fly).	Interval between feedings (average per fly).	Time required to complete meal on healthy host (average per fly).	
					Seconds.	*Seconds.*	*Mins.*	*secs.*
1	127	Numerous	68	30	20	40	3	3
2	127	Swarming	87	20	45	120	2	30
3	127	Moderate	118	15	15	52	3	30
4	127	do	115	12	15	45	3	20
5	127	Numerous	130	12	18	45	4	00
6	127	do	126	15	20	25	2	45
7	127	Moderate	111	21	20	30	3	00
8	127	Numerous	116	26	30	120	1	00
9	127	Moderate	81	8	20	40	1	30
10	127	do	95	8	15	20	1	45
11	127	do	106	6	15	45	1	35

Negative results were obtained in all of these experiments.

Fifteen to thirty seconds are consumed by the fly in inserting the proboscis to the depth of the bulb of the labium and to the stage of aspiring the blood. Much depends on the strength and rigidity of the labium in this regard, for, in a hungry fly newly emerged, as much as two minutes is sometimes required before the proboscis is sufficiently embedded to start the blood flow either by capillarity or suction.

Ordinarily, under conditions of an experiment, if the interruption takes place after two minutes, renewed feeding on the second host does not take place for twenty minutes or more; on the other hand, some flies may become engorged in twenty

to thirty seconds. The flies used in this series were laboratory bred, applied on a single occasion only, with the exception of those in experiments 10 and 11, wherein the flies had been used previously in experiment 9. (Table VI.)

Two attempts at mechanical transmission by interrupted feeding were made with horses as the second hosts. The method pursued was the same as before, but the flies employed were not laboratory bred. It was aimed in this series to exaggerate the normal conditions as much as possible by using as virulent a strain of surra as could be obtained and by transferring the infected flies to the weakest animals available. Unfortunately the number of flies employed was not adequate, due mainly to the great length of time required to feed them and the desire to save needless suffering of the horses strapped to the operating table. Four to five hours were required to complete each experiment.

The experiments were performed in the screened operating room of the laboratory where the horse was strapped to the operating table and bitten as rapidly as the flies could be transferred from the infected guinea pig on the adjoining table. As many as 4 flies could be fed simultaneously on the horse in this manner. Individual tubes were used to hold each parasite. On July 1, 1912, horse 275 was bitten by 25 flies which were applied at intervals of from ten seconds to two minutes after contaminating their labiums with blood of guinea pig C. The time required to complete the meal varied with the individual flies from forty seconds to five and one-half minutes.

On the following day, July 2, horse 279 was similarly treated with 38 flies. These were fed for from twenty to thirty seconds on surra guinea pig A, which had a maximum infection at this time. The flies required from thirty seconds to six minutes to become completely engorged on the second host.

Details of the work appear in the following table:

TABLE VII.—*Experiments and interrupted feeding of Stomoxys on horses.*

Infected guinea pig used.	Average number of trypanosomes present in blood per field.	No. of horse employed.	Number of flies applied.	Length of time fed on surra host (average per fly).	Interval between feedings (average per fly).	Time required to complete meal on the horse (average per fly).	Duration of experiment.
				Seconds.	*Seconds.*	*Minutes.*	*Hrs. mins.*
C	50-60	275	25	20	30	3.5	4 20
A	65	279	38	25	25	4.0	5 00

Guinea pigs C and A used in Table VII received their infections from subcutaneous inoculations of the peritoneal fluid of guinea pig 128 which was a mate to guinea pig 127, receiving the disease from an emulsion of infected house flies. Death of 128 occurred on the fifty-second day after the injection. Guinea pig C died forty days after inoculation. Numerous trypanosomes were seen in a drop of its heart's blood. Guinea pig A was also positive at death fifty-four days after injection. Blood taken from the spleen was found swarming with trypanosomes.

After the experiments the horses were replaced in the fly-screened stable where they were held for thirty days for examination. During this period no symptoms of surra developed, after which blood was drawn from each, and inoculated into guinea pigs. The horses were further tested as to susceptibility to the disease by the inoculation of infected blood from a sick guinea pig. Trypanosomes were recovered from horse 275 on August 17, 1912, and from horse 279 on the same day. The characteristic febrile changes occurred in both horses beginning on the evening of August 17, 1912.

MECHANICAL TRANSMISSION BY SUCCESSIVE INTERRUPTED FEEDINGS.

In this series of experiments 3 guinea pigs were subjected to interrupted bites of infected flies for from six to eight days. Guinea pig 177 was bitten from June 20 to June 27 by 40 flies, guinea pig 187 was bitten from June 28 to July 5 by 28 flies, and guinea pig 129 received the bites of 206 flies from June 30 to July 5. Laboratory-bred flies were not used in the first two experiments as the breeding jars were not productive at this time. In the third experiment laboratory-bred flies were used daily during the course of the experiment.

In the experiment with guinea pig 129 as the host, the flies were applied in the six days during thirty-two hours, which represents a fairly constant infestation by infected flies. The precaution was taken in this instance, as in the other experiments, to distribute the feeding area over various parts of the body in order to abrade the skin as little as possible each day.

The usual animal stock was employed as well as the method of feeding individual flies from test tubes. A fresh collection of flies was used daily.

TABLE VIII.—*Representing successive interrupted feeding from infected to healthy guinea pigs.*

Date.	Infected animal used.	Condition of blood of donor relative to trypanosomes.	No. of healthy guinea pig used.	Number of flies applied.	Length of time fed on surra host (average per fly).	Interval between feedings (average per fly).	Time required to complete meal on healthy host (average per fly).	Duration of experiment.
					Seconds.	*Seconds.*	*Mn. secs.*	*Hr. mins.*
June 20	127	Swarming	177	6	35	35	3 30	1 00
June 21	127	Numerous	177	5	30	60	4 00	1 00
June 22	127	Swarming	177	5	20	60	2 00	30
June 23	127	do	177	5	20	10	3 00	35
June 24	127	do	177	5	25	30	3 30	20
June 25	127	Numerous	177	4	25	5	2 30	40
June 26	127	Swarming	177	5	25	15	2 00	30
June 27	127	do	177	5	20	30	3 00	25
June 28	C	Numerous	187	4	20	10	2 30	20
June 29	C	Scanty	187	4	20	50	2 00	25
June 30	C	Moderate	187	4	20	40	2 00	15
July 1	C	do	187	4	20	20	2 00	20
July 2	C	Numerous	187	3	25	25	2 00	25
July 3	C	Swarming	187	3	20	20	2 00	15
July 4	C	do	197	3	25	30	3 00	15
July 5	C	Numerous	187	3	20	35	3 00	10
June 30	A	Scanty	129	31	15	50	3 00	6 00
July 1	A	Moderate	129	28	15	25	3 00	5 00
July 2	A	Numerous	129	25	20	35	1 30	3 00
July 3	A	Scanty	129	20	20	30	2 00	1 00
July 4	A	Moderate	129	42	20	25	3 00	8 00
July 5	A	do	129	60	20	30	1 30	8 00

The guinea pigs employed in the first two experiments were negative up to August 1, 1912, when they were inoculated with blood of a guinea pig positive for surra. Both reacted in the usual manner, showing numerous trypanosomes on the fifth and sixth days, respectively. Both were alive and positive for surra up to August 26, 1912.

A positive result was obtained in the third experiment with guinea pig 129 which showed scanty trypanosomes on July 11, six days after the last lot of flies was applied to it. On the following day trypanosomes were present in moderate numbers, and two guinea pigs were inoculated with its blood. These showed trypanosomes on the seventh and eighth days after inoculation. Guinea pig 129 was examined daily until August 11, 1912, during which time trypanosomes were seen in numbers from

moderate to numerous. The animal died August 12, 1912, when its heart's blood was used to test the disease susceptibilities of the 2 horses, 275 and 279, used in a previous experiment. Trypanosomes in moderate numbers were recovered from these animals August 17, 1912.

DURATION OF THE INFECTION IN THE PROBOSCIS OF STOMOXYS.

Dutton, Todd, and Hanington(34) found that red cells and *Tr. gambiense* were almost always present in the labium of *Gl. palpalis* up to ten minutes after feeding. The longest period in which trypanosomes were found in the labium was one and three-fourth hours, and red cells seven and one-half hours.

As far as can be determined no authentic records exist in which *Stomoxys* has been investigated in this connection.

An effort was made to determine how long the proboscis of *Stomoxys* can retain trypanosomes. Six experiments were performed with 2 animal inoculations in each. The method employed was to feed laboratory-bred flies on an infected guinea pig and after certain intervals stupefy the insects and immediately sever the head from the body. With another set of instruments the proboscis was dissected and at once emulsified with normal saline solution and injected on a cotton pledget into a subcutaneous pocket of the abdomen of a guinea pig previously etherized. This was followed by a similar mode of inoculation, using disinfected instruments, with the macerated abdomens. The thorax was invariably discarded. The interval of time between the withdrawal of the insect's labium from the infected animal and the dissection of the mouth parts was carefully noted.

In two experiments the flies were purposely interrupted in the biting process, and in the other trial the flies were allowed to complete the meal unmolested. In the cases of interrupted feeding one-half to three minutes were allowed for each fly to insert the proboscis to the depth of the bulb of the labium, the feeding being interrupted at a stage when there ensued a barely perceptible inflation of the abdomen.

When permitted to feed uninterruptedly it has been noticed that this insect sucks its food cleanly, no residue adhering to the labellum of the mouth or to the labium externally. In one instance (experiment 5) it was observed that in 2 flies chloroformed prior to dissection droplets of fresh blood oozed from the proboscis. The abdomens of these flies were fairly engorged with blood. It is suggested that the phenomenon observed was a regurgitation of blood from the pharynx into the labium, resulting either from the engorgement of the stomach or from the

effects of the chloroform on a full stomach. The latter is regarded as the more plausible explanation.

Dutton, Todd, and Hanington[34] write: "It was also found that flies (*Gl. palpalis*) caught after they had fed on an infected animal, frequently regurgitated a drop of blood as large as a pin's head, which was full of parasites, many of them identical in form with those ingested. This was observed up to twenty-eight hours after infection." The significance of regurgitation as a means of transmission is noted.

Koch[44] also found that, by pressure of the proboscis, trypanosomes could be obtained from the labium of an infected *Glossina*.

Table XV contains data of experiments concerning the point under discussion.

TABLE XV.—*Infectivity of dissected flies.*

No. of experiment.	Time of completed or interrupted feeding.	Number of flies used.	Interval prior to dissection of flies.	Guinea pig receiving proboscides.	Guinea pig receiving abdomens.	Results of inoculation and fate of guinea pigs.
			Minutes.			
1	Interrupted ½ to 3 minutes.	3	1.5	R	U	R negative. U positive tenth day.
2	Complete, 5 minutes...	1	0.5	T	S	T positive twelfth day, dead thirtieth day. S positive twelfth day, dead thirty-second day.
3	Complete, 40 seconds to 1½ minutes.	5	0.5	103	102	103 positive ninth day. 102 negative.
4	Interrupted 30 seconds.	6	5.0	121	117	121 negative, dead twenty-fifth day. 117 negative, reacted positive to inoculation of subsequent experiment.
5	Complete, 1 to 7 minutes.	5	0.5	113	105	113 positive tenth day. 105 negative.
6	Complete, 1 to 2½ minutes.	5	5.0	101	28	101 negative. 28 positive ninth day, dead twenty-fourth day.

Attempting to draw a deduction from the above data, it is found that the surra organism remains in the proboscis for thirty seconds, but disappears in one minute and thirty seconds after the infective meal. The guinea pigs inoculated with the abdominal contents of the flies serve as a control of the experiments. The table demonstrates also that the proboscis of a fly interrupted in its feeding, under the conditions stated, does not appear to be infective. These experiments are by no means conclusive.

It was thought desirable to make an effort to ascertain whether or not infection by direct transmission is due to any external contamination. In other words, in what manner other than

through feeding can infection be transported by the fly. This would involve the introduction from without of infective materials into the punctured skin of the host. Such external factors might include excretory contamination, contamination from the insect's pulvillus, and more remotely that from hairs of the insect's body or that from the wings.

A series of experiments was performed to decide whether or not the wound caused by the fly's proboscis was suitable for the entrance of infective material. In these trials a varying number of bred *Stomoxys* flies were induced to bite healthy guinea pigs whose skin was thoroughly shaved but not abraded. A generous platinum loopful of blood freshly drawn from the ear of a surra guinea pig was rubbed into the fresh bite immediately after each fly was withdrawn. In this series of experiments the infected blood used was taken from guinea pig T, which reacted to inoculation of a single proboscis of a fly fed on a surra animal. Guinea pig T, which is accounted for in Table XV, was used here when its blood swarmed with surra trypanosomes. In every instance, after a lot of flies were fed, the area of the skin covered by the inverted test tube was vaccinated with a saline solution containing heavily infected surra blood. The following table contains information as to the work done on this subject:

TABLE XIV.—*Results of rubbing infected blood into wounds caused by proboscides of flies.*

Number of bites.	Guinea pig used.	Results.
8	121	Negative.
20	105	Do.
7	102	Do.
13	103	Do.*

* Reacted six weeks later to inoculation of surra blood.

To what extent the fæces from infected flies are contaminated has not been systematically determined. One experiment to determine the range of this infection was tried up to eighteen hours with fæces of infected flies. According to the results, although degenerative forms were detected microscopically, the injected material was devoid of infective trypanosomes. The experiments were concluded in each case with a negative result. For the present, therefore, with the evidence at hand, the possibility of infection by fly dejecta rubbed into the bitten skin is considered as nil.

The possibility of infection being carried by the fly's pulvillus was tested by using flies (*Musca domestica*), not bloodsuckers, whose pulvilli were as large as, or larger than, those of *Stomoxys*. Both monkeys and guinea pigs were used in these tests. The tests were made more conclusive by using large numbers of flies in bottles applied to the monkeys and a smaller number in large glass tubes on guinea pigs.

In the first of the monkey experiments, 30 laboratory-bred *Stomoxys* were applied to the tail of the healthy animal immediately followed by 50 flies (*Musca*) from a separate bottle, which previously had been applied to the abraded tail of a surra monkey. Twenty minutes were allowed the *Musca* to carry the infected contents of feet and mouth parts into wounds left by the clean *Stomoxys*. In the other experiment with the monkey, 200 *Stomoxys* and 250 *Musca* were employed. The 3 guinea-pig experiments were performed in a similar way with fewer flies.

All 5 experiments resulted negatively, the animals being used later for other purposes. One of the guinea pigs subsequently reacted to an inoculation of infected blood.

THE RELATION OF NONBITING FLIES TO STOMOXYS IN CONTAMINATIVE INFECTIONS.

In considering the relations existing among flies of the family Muscidæ and their parasitism, a peculiar phase is brought to light. I was curious to learn why such an abnormal percentage of nonbiting flies was generally found in collecting insects from domestic animals. In an examination of extensive collections made with a net swung over the backs of the animals, the majority of the nonbiting flies were found to contain blood-engorged abdomens. These when dissected and examined microscopically showed mammalian blood to be the principal food constituent.

A quiet bullock was selected for closer observation. Some 150 to 200 flies, mostly muscids, were seen to collect on him. Many hundreds of dung flies, including house flies, were scattered about on the floor of the stall, and an occasional one of these was seen to join the others on the host's body.

In a short while my attention was attracted to the peculiar grouping of the ectoparasites; groups of 2 to 4 and 5 prevailed. On closer inspection the group was found invariably to consist of more than one species, a *Stomoxys* usually providing the central figure. Where this species was lacking it was found that the group fed from a common area with the heads of the individuals in close contact. The food of the latter was found to be a

droplet of freshly exuding blood, and among these often not an individual belonged to a species with a piercing mouth; they consisted principally of house flies. Other groups of flies surrounding the *Stomoxys* attracted attention by the fact that while it fed the rest waited. The latter gave evidence of great impatience and eagerness in the movements of nudging one another and colliding with the *Stomoxys*, apparently making efforts to dislodge it. The *Stomoxys* having been satisfied, the other flies pounced upon the feeding spot where a well-rounded blood-drop trickled, and lapped the blood as it oozed from the wound. In a moment the group disbanded with abdomens more or less reddened and distended, the individual either flying off the host to rest or joining another biting *Stomoxys*. In many instances the *Stomoxys* was accompanied by a single fly which hovered about it in a highly provoking fashion. Several minutes elapse, however, before the *Stomoxys* is fully engorged and the blood is left to the disposal of the secondary passive parasite.

It has been noticed that even other bloodsucking flies found on cattle often take advantage of the action of the more powerful proboscis of the *Stomoxys*. *Lyperosia* was found to await its turn with other nonbiting flies for the free-for-all blood feast. This was noted in two instances on the pachydermic skin of the carabao where the relatively feeble mouth of this diminutive muscid was a decided handicap. In this instance, especially among grazing carabaos, *Lyperosia* will hover in a swarm above a lone bloodsucking *Stomoxys*. To be sure the *Lyperosia* will probe for blood on its own initiative, as will smaller flies like some of the Chironomidæ, but apparently when so much energy is required on a thick-skinned animal like the carabao, blood in the readily available form provided by the *Stomoxys* will be imbibed readily. *Lyperosia* was never observed to provide blood for other hawking dung flies, although this probably occurs. Another haustellate muscid, a *Philoematomyia*, was observed to feed independently of other flies. Although its mouth is not strictly a piercing organ, the epidermis is penetrated, blood being drawn to the surface of the skin and sucked cleanly.

In order to secure additional evidence of the blood-feeding habits of the nonbiting flies, experiments were conducted to determine the relationship of the common house fly, *Musca domestica*, to *Stomoxys* as a harborer and carrier of trypanosomes. In these experiments it was aimed first to prove that *M. domestica* can harbor within its body infective trypanosomes. The normal protozoan fauna of these flies was not taken into consideration,

as no great stress was laid on microscopical findings. Emulsions from dissected flies fed on surra blood did, however, show organisms resembling *Trypanosoma evansi*. In the following experiments house flies were employed which had emerged April 23 and 24, 1912, from laboratory cultures. A large number of these were applied to the abraded tail of a surra-infected monkey, and three hours later an emulsion from 3 flies was found swarming with trypanosomes. At this time 20 flies of the lot were inoculated into 2 guinea pigs, the abdominal contents only being used. Stained smears of the solution that was inoculated revealed the presence in moderate numbers of organisms indistinguishable from *Tr. evansi*.

The two inoculated guinea pigs, 127 and 128, were found to be infected on the seventh and eighth days respectively. Both showed trypanosomes on numerous occasions and also at death, which occurred on the sixty-fifth day after inoculation in the case of No. 127, and on the fifty-second day in the case of No. 128.

Two hundred fifty flies of this lot were utilized in carrying through the following experiment. Prior to infection of the house flies, 200 *Stomoxys* were placed on a surra monkey's tail (which had not been previously abraded). After thirty minutes the majority of the flies had fed, and then a fresh bottle, containing the 250 *Musca*, was substituted. The house flies fed ravenously on the blood brought to the surface by the probes of the first flies. When a large number of the *Musca* showed partly blood-engorged abdomens, which occurred in fifteen minutes, they were withdrawn and applied to a healthy monkey after 200 hungry, newly emerged *Stomoxys* were turned loose in the same bottle, both species being then applied to the tail of the fresh monkey. Here the attempts to simulate natural conditions were successful; the *Musca* fed after the *Stomoxys*, lapping the fluid from punctures made by the latter. The flies were not disturbed until all were apparently satisfied, which was a matter of forty minutes.

In a second experiment 30 laboratory-bred flies were applied to a healthy monkey and 25 bites were recorded. Immediately 50 *Musca* which had fed from a fresh wound on a surra animal were substituted for the *Stomoxys*. In twenty minutes 30 to 40 of the *Musca* had lapped blood from the healthy monkey's tail. Full opportunity was given them to carry infected material on labella and pulvillus into the wounds presented. Three other experiments were conducted with guinea pigs as hosts. In one, 40 *Musca* accompanied 20 *Stomoxys;* in another, 14 *Stomoxys* and 80 *Musca* were used; and in the last, 20 *Stomoxys* were

followed by 60 *Musca*. The flies were used in much the same manner as in the preceding experiments, the *Musca* feeding, after contamination, from the wound made previously by healthy *Stomoxys* on guinea pigs free from disease.

All of these experiments were followed by negative results.

There is demonstrated at least, that a wound caused by the mouth prick of *Stomoxys* is not suitable for the entrance of surra-infected material transported by the mouths and feet of many house flies. A logical sequel to this series would be to transfer the muscids, after they were supplied with infected blood from *Stomoxys*-probed wounds, to open sores and scratches found on work animals. This work is under way at the present time.

The probability of success by this method is indicated by the experiments of the following writers:

Musgrave and Clegg[23] transmitted surra to healthy animals through the agency of house flies.

Darling,[56] in the Panama Canal Zone, recently conveyed *Tr. hippicum* to the mule by means of 18 house flies.

THE CYCLICAL DEVELOPMENT OF TRYPANOSOMA EVANSI IN STOMOXYS CALCITRANS.

The literature is abundantly supplied with theories and conjectures in regard to the development of trypanosomes within the body of the intermediate insect host. Aside from the monumental work of Kleine, Bruce, and his collaborators, on the development of trypanosomes in tsetse flies, we possess little definite knowledge. In regard to *Stomoxys* as a definitive host, the experiments that have been performed are far from satisfactory, the most serious obstacle encountered being the inability of various workers to keep this species of fly alive long enough for complete investigation. The views of various investigators are cited as follows:

Austen[35] finds from the evidence submitted, no indication that trypanosomes ingested by *S. calcitrans* pass through a developmental cycle and they apparently disappear within forty-eight hours.

Manson[40] notes that *Stomoxys* probably acts as the definitive host for *Tr. evansi* and *Tr. equinum*.

Schat[14] is apparently convinced that *Stomoxys* serves as the definitive host of *Tr. evansi* and that surra parasites propagate in the body of this fly.

Ziemann[41] thinks that *Tr. vivax* is transmitted by *Stomoxys* which acts as a definitive host, the trypanosomes multiplying in its body. No experimental evidence is cited.

Leese[42] discusses cases of camel surra in India. He considers the mechanical theory of transmission perfectly adequate, and that a life cycle of the development of the trypanosomes in the biting flies, *Stomoxys* and others, is not tenable except by analogy.

Gaiger[29] in speaking of *Stomoxys* and other flies mentions that no

experiments have yet been carried out in India, on the lines of those of Kleine and Bruce in Africa, to show that, in an odd fly or two, trypanosomes may survive as in a culture medium and that possibly a sexual cycle may occur.

Baldrey[43] believes with Schat that there is evidence of a cyclical development in flies infected with surra. He attempts to show that the development of *Tr. evansi* in the fly is probably completed through a mammal. He finds the trypanosomes in the fly quickly dying, and sees a spore stage which is incapable of reproducing the disease. This suggests to him that direct transmission by the fly is not the usual method since it is rare to find trypanosomes in the proboscis of the fly immediately after feeding. The longest time *Stomoxys* is kept alive for his experiments is ten days. All feeding experiments were negative. Injections of flies were found positive within twenty-four hours after infection.

Leese[21] fed wild *Stomoxys*, caught on surra animals, on a white rat after five to twenty-one days of capture from infected hosts. The result was negative for evidence of a cycle of development. Three experiments were conducted with *S. calcitrans* in interrupted feedings of one-half- to three-minute intervals. Fifteen flies transferred from a white rat to a healthy guinea pig gave a negative result. Ten flies fed from surra animals in the field transferred to a white rat proved negative. Ten flies from an infected white rat to a healthy white rat produced a positive result when fed—1 on the first day, 2 on the second day, and 7 on the third day. The flies were applied from tubes inverted over the animals.

Koch[44] in a measure anticipated Kleine and Bruce in their classical studies on cyclical development in insects. Koch's investigations in sleeping sickness with *Glossina* "led him to conclude that the flies did not transmit the disease by carrying the blood directly from an animal to another as is usually supposed, but that the trypanosomes pass through a developmental stage in the fly."

Kleine[45] with the use of laboratory bred *Glossina* was able to show convincingly a distinct cycle of development of *Tr. gambiense* in the flies.

Bruce and others,[46] working with *Tr. gambiense* and *G. palpalis*, found that from a lot of 60 flies 1 survived on the seventy-fifth day after infection and after previously infecting a monkey reproduced the disease by subcutaneous inoculation. A tiny drop of the emulsion was sufficient. Salivary glands, besides other organs, were infective in this fly.

Bagshawe,[47] criticizing Baldrey's paper, says: "Such transmission experiments as these should be continued with a large number of flies and, if possible, for longer periods. In the case of the rat-flea and the tsetse-fly, only a small percentage get a permanent infection with trypanosomes; hence the odds against experiments with single flies succeeding are considerable. No reliable evidence of a cycle of a pathogenic trypanosome in *Tabanus* or *Stomoxys* will be obtained from a study of the parasites found therein after infected feeds till the flies have been bred."

Speaking of the carriers of surra in this connection, the author last quoted remarks: "As it has been pointed out before, the first essential is to breed and keep in captivity the flies which are under suspicion, and until this has been done we shall remain in uncertainty whether there is or is not a special development of the surra organism in the invertebrate host. Our knowledge of the life history of the African species of trypanosomes makes it very probable that there is."

EXPERIMENTS ON CYCLICAL DEVELOPMENT.

The first of this series of experiments was conducted with a small number of wild flies at a time when bred flies were not available. The experiment was followed out to completion mainly to acquire a technique for keeping flies alive under laboratory conditions. In this respect the tests were successful. The flies were kept individually in glass tubes and fed daily until the last fly of the original lot died. Fourteen flies were used on the first day after infection by one bite per fly on a heavily infected guinea pig (45). The duration of the experiment was sixty-seven days from the initial infective feeding. The sole survivor was too enfeebled to feed on the sixty-eighth day, when it was inoculated in a subcutaneous pocket of a healthy guinea pig. Fifty days elapsed without any infection resulting; therefore, it was assumed that the feeding experiments were negative.

Two other experiments were tried with laboratory-bred flies with the object of using as many flies as possible at once. Large bottles were utilized into which the monkey's tail was introduced to be fed to the flies. The stock of flies rapidly diminish until the twenty-eighth and thirtieth days.

Prior to the beginning of the first experiment (Table X), the flies were applied for two days on monkey A, which upon both occasions had only a very few trypanosomes in each field of blood examined. Trypanosomes were seen in emulsions of 3 flies of this series on the second day of biting the infected monkey.

The flies used in the second of these experiments (Table XI) were fed twice on the blood of surra monkey R. At each feeding a moderate number of trypanosomes were present in its blood. A single fly which was injured after feeding on monkey R showed a large number of surra-like flagellates in an emulsion of its intestinal tract.

In one instance the survivors of the experiment were inoculated into guinea pigs to test the presence of infective organisms. These animals did not react. The other experiment ended on the thirty-first day with the death of the last 2 flies of the original 75 flies which had been applied to guinea pigs at the beginning of the experiment.

None of the guinea pigs and monkeys employed in the series was used for other purposes until forty-four days after the completion of feeding by the flies. During this time the animals were examined at convenient intervals, but no indications of the infection were encountered.

A final experiment to complete the series was made one month later with laboratory-bred *Stomoxys*, kept individually in suit-

TABLE X.—*Successive feeding of Stomoxys from infected to healthy animals.*

Length of time after the last infective meal.	Healthy host employed, monkey—	Number of flies applied to healthy animal.	Number of flies biting healthy animal.	Length of time after the last infective meal.	Healthy host employed, monkey—	Number of flies applied to healthy animal.	Number of flies biting healthy animal.
Days.				*Days.*			
1	C	190	182	16	C	23	22
2	D	134	115	17	D	22	19
3	E	123	114	18	F	22	21
4	F	113	100	19	B	20	20
5	G	93	93	20	K	18	16
6	H	70	68	21	L	14	13
7	I	65	60	22	N	14	12
8	J	56	53	23	O	14	12
9	K	52	49	24	P	14	10
10	N	49	45	25	T	14	14
11	O	46	35	26	U	12	11
12	P	43	28	27	X	10	7
13	Q	32	24	28	G	6	5
14	S	24	21				
15	T	24	22				

TABLE XI.—*Successive feeding of Stomoxys from infected to healthy animals.*

Length of time after the last infective meal.	Healthy host employed, monkey—	Number of flies applied to healthy animal.	Number of flies biting healthy animal.	Length of time after the last infective meal.	Healthy host employed.	Number of flies applied to healthy animal.	Number of flies biting healthy animal.
Days.				*Days.*			
1	M	60	48	16	Monkey E	8	6
2	N	60	48	17	Monkey C	5	4
3	O	54	56	18	Monkey S	4	4
4	P	44	28	19	Guinea pig 89	4	4
5	D	36	36	20	Guinea pig 84	4	4
6	H	34	20	21	Guinea pig 111	2	2
7	J	29	29	22	Guinea pig 92	2	2
8	K	26	25	23	Guinea pig 99	2	2
9	L	23	23	24	Guinea pig 50	2	2
10	2N	18	13	25	Guinea pig 110	2	2
11	2O	18	16	26	Guinea pig 131	2	2
12	2P	14	12	27	Guinea pig 88	2	2
13	G	12	9	28	Guinea pig 88	2	2
14	2H	12	11	29	Guinea pig 88	2	2
15	2J	9	7	30	Guinea pig 88	2	2

TABLE XII.—*Successive feeding of Stomoxys from infected to healthy animals.*[a]

Interval after feeding on infected animal.	Healthy animal used, monkey—	Number of flies applied.	Interval after feeding on infected animal.	Healthy animal used.	Number of flies applied.	Interval after feeding on infected animal	Healthy animal used.	Number of flies used
Days.			*Days.*			*Days.*		
1	C	87	19	J	53	37	8	14
2	D	79	20	M	49	38	9	13
3	G	90	21	N	47	39	9	12
4	L	85	22	O	45	40	9	9
5	M	83	23	P	43	41	11	4
6	N	81	24	S	42	42	11	2
7	P	80	25	1	40			
8	S	77	26	2	40			
9	1	73	27	2	38			
10	2	72	28	2	37			
11	3	70	29	3	37			
12	4	67	30	3	35			
13	8	66	31	3	33			
14	9	65	32	4	31			
15	10	61	33	4	30			
16	11	58	34	4	30			
17	C	58	35	8	28			
18	G	55	36	8	20			

[a] Guinea pig A was used to feed the 90 flies of this series for three days prior to their application upon the first healthy monkey. During the three days the blood of guinea pig A was richly infected with trypanosomes. In emulsions made, 3 out of 4 flies examined showed tremendous numbers of trypanosomes indistinguishable from those seen in the host's blood.

INOCULATION OF FLIES FED ON INFECTED ANIMALS.

To complete the discussion of the cyclical development of the trypanosomes within the fly, it is necessary to refer to the length of time flies remain infected after imbibing infective material. Although, because of the uniformly negative results obtained in many transmission experiments, this information remains of no immediate practical value, it is included because the literature on this subject is regarded as tending to mislead by magnifying its importance.

The length of time the infection is held in the insect certainly is of prime significance where the infection is produced normally through crushing of the intermediate host or through fæcal contamination. In both instances the transmission is consummated by injection of the contaminative material into an abrasion produced by the insect's mouth parts.

The presence of surra trypanosomes in the fly has been demonstrated up to forty-eight hours by several authors. The organism in all cases has been found in the intestinal tract and never within the salivary glands and rarely in the proboscis. In some instances the contents of the intestines of an infected fly have given rise to the disease through inoculation into animals, while in others the writer appears contented with the microscopical findings. From a survey of the literature at hand it appears that the infection was never reproduced by the former method beyond twenty-four hours.

Bruce[48] states: "a *Glossina* fly, a few hours after feeding on an infected animal, crammed with blood showing active haematozoa under the microscope, if minced up and injected under the skin of a susceptible animal, fails to give rise to the disease (nagana)."

Bruce and others[49] note, in an attempt to ascertain the number of tsetse flies infected with *Tr. gambiense*, that in one case in which no trypanosomes were found in 12 flies examined, when their combined contents were injected into a healthy monkey, sleeping sickness resulted.

Bruce and others[50] injected monkeys with a single fly (*Glossina palpalis*) one day; 2, 3, and 4 flies two days after an infected feed. These caused sleeping sickness. Between the second and twenty-fourth days after infection 249 flies were inoculated, in all with negative results, although 15 of these flies proved microscopically to be swarming with living trypanosomes at the time of inoculation.

Martini[32] found trypanosoma in *Stomoxys calcitrans* twenty-three hours after an infected meal. The organisms were not observed on the day following, and they were apparently digested.

Nabarro[18] cites a case where trypanosomes from a mule in East Africa were found in the stomach of *Stomoxys* as long as thirty hours after a meal of infective blood.

Dutton, Todd, and Hanington[34] found trypanosomes (*Tr. gambiense*) unchanged in the gut of *Stomoxys* up to twenty hours after feeding on a sick horse. Also on two occasions they found fusion forms of the trypanosome in a fly fed eighteen hours previously.

Nabarro[18] states that the organisms of sutoko, a trypanosome disease of the nagana group, were found active in the stomach contents of *Stomoxys* up to twenty-four hours after infection.

Bruce[51] found in Uganda scanty trypanosomes in the proboscis of *Glossina morsitans* fed forty-six or fewer hours previously on a nagana-infected animal.

Nabarro,[18] referring to fly trypanosomiases of Uganda, recalls that organisms remain active for a longer time in the stomach of *Stomoxys* than in that of *Gl. palpalis*. Feeding experiments with eight- and twenty-four-hour intervals from infected to healthy animals were negative in *Stomoxys*. *Stomoxys* was positive in direct transmission in interrupted feeding with dogs.

Nabarro[18] writes that trypanosomes taken from an infected monkey (Abyssinian strain of trypanosomiasis) by *Stomoxys* remained active in the stomach for twelve hours, in that of *Gl. palpalis* five and one-half hours.

The following table shows the length of time during which *Stomoxys* was found to harbor the surra organisms. The few laboratory-bred flies that could be spared for the purpose were fed on a virulent strain of guinea-pig surra and injected into healthy guinea pigs at stated intervals. When the fly was to be kept beyond twenty-four hours, it was fed a few minutes each day on an animal not included in this experiment. In every case the entire fly was inoculated.

TABLE XIII.—*Results of inoculating flies fed on an infected host.*

Length of time of infective meal.	Time elapsing after feeding on infected host.	Number of flies injected.	Trypanosomes present or absent at the time of injection.	No. of the test guinea pig used.	Results of inoculation.
Minutes.	*Days. hrs.*				
3	(a)	1	Present	37	Positive on eighth day.
					Positive on thirty-eighth day at death.
5	6	1	.....do.	35	Positive on sixth day.
					Positive on forty-second day at death.
4	18	1	Few alive	50	Animal negative.
5	18	1	Few active	168	Do.
4	24	1	Absent	91	Do.
5	24	3	.....do.	32	Do.
6	30	3	.....do.	33	Do.
7	48	2	.....do.	130	Do.
9	54	3	.....do.	126	Do.
8	29 00	6	.....do.	73	Do.
				74	
10	68 00	1	.....do.	34	Do.[b]

[a] Used immediately.

[b] A wild fly used for successive feedings on guinea pigs.

A check on the experiment in the use of this species of fly is offered in a former experiment by an inoculation of a forty-two-hour infected mosquito, *Stegomyia fasciata*. Here a positive result was obtained.

THE QUESTION OF HEREDITARY TRANSMISSION.

The experiments next described were performed with the purpose of eliminating all possible avenues through which infection might possibly be transmitted by means of the fly.

Sergent, Ed. & Et.(33) recount experiments with Algerian trypanosomiasis in which feeding tests, with young ticks hatched from eggs laid by adults removed from heavily infected animals, were negative.

Dutton, Todd, and Hanington (34) write: "It is possible that the progeny

of infected tsetse flies are capable, or are alone capable, of transmitting the trypanosomes." (*Tr. gambiense.*)

Fraser and Duke(53) give detailed results in feeding hundreds of laboratory-bred *Glossina* thirty days upon healthy monkeys to determine if a hereditary transmission of *Tr. gambiense* existed. Only negative results were obtained.

Kleine and Taute(54) used thousands of tsetse flies bred from pupæ without encountering a single instance of hereditary transmission of the sleeping-sickness trypanosome. They view with skepticism the finding of trypanosomes in the eggs of infected flies.

Kleine(55) found that none of the experiments with *Gl. morsitans* and *Tr. gambiense* supported the theory of hereditary transmission of the trypanosome. Hundreds of flies were used.

Bruce and others,(49) in experiments with laboratory-bred *Glossina* and sleeping sickness, obtained no evidence of hereditary transmission in the use of several hundreds of flies.

Baldrey(43) expresses a belief in the theory of transmission by heredity. His observations relate to *Stomoxys*, Tabanidæ, and surra.

Minchin writes that, so far as it is permissible to draw general conclusions from experiments which yield negative results, it appears that trypanosomes are not transmitted from parent to offspring in insect carriers. The experiments referred to were carried on in 1911 with fleas and *Trypanosoma lewisi*, by Minchin and Thomson. These authors sum up their experience thus: Experiments on a large scale had been done to see if transmission can take place hereditarily in the flea, that is to say, whether the offspring of the infected flea themselves may be infected. These were continued for some months, but have always been negative.

Aside from the biological significance of hereditary transmission, there is involved a practical problem for the laboratory worker. If pathogenic trypanosomes were inherited, the same objection for employing wild flies would hold for laboratory-bred flies whose parents were wild. Under these circumstances the newly emerged laboratory-bred flies would need to be proved surra-free prior to their experimental use.

In the present series of experiments the aim was, first, to test the possibility of the transmission of surra from fly to fly through the egg to the new generation, and, second, to simulate the possibility of conveyance of the trypanosomes through the imago of flies, the larvæ of which were fed on infected material.

In the first of these experiments the flies used were the progeny of flies fed, previous to egg laying, on surra-infected guinea pigs for periods of from three to five days. The eggs were laid from February 27 to March 15, 1912; within a day after emerging the new flies were fed on a healthy guinea pig. Daily additions to the number of flies fed were made as fast as they emerged. The feedings were conducted during nine days, at first with 7 flies and later with 25 flies. Data on the subject appear in the following table:

TABLE XVI.—*Feeding the progeny of infected flies.*

Date.	Number of flies applied.	Total length of time flies were fed.	No. of animal used.
		Hours. mins.	
Mar. 21, 1912	7	36	Guinea pig 68.
Mar. 22, 1912	8	41	Do.
Mar. 23, 1912	7	36	Do.
Mar. 24, 1912	16	1 1	Do.
Mar. 25, 1912	19	1 29	Do.
Mar. 26, 1912	22	1 37	Do.
Mar. 27, 1912	23	1 49	Do.
Mar. 28, 1912	25	1 55	Do.
Mar. 29, 1912	25	2 3	Do.

The result of this experiment with guinea pig 68 was negative. This animal has been used since for surra inoculation to which it reacted positively August 4, 1912.

A second experiment of this type was carried out with the progeny of flies fed on a surra horse. The horse was kept in a fly-screened stall for six days during which time flies were permitted to feed undisturbed. Several hundreds of the flies were removed from the stall and placed in a jar with horse manure. Seventeen days later new flies emerged, 75 of which were selected for feeding on a guinea pig. The flies were fed daily for eight days when the animal was kept under observation for forty-five days, after which time the experiment was judged to be negative.

Surra organisms have never been encountered microscopically in numerous lots of eggs laid by infected flies nor in emulsions of larvæ developing from eggs deposited by surra-fed adults.

On May 9, 1912, this was tested in a more convincing way by inoculating material of this sort. With laboratory-bred flies as the parents, 30 larvæ, the progeny of 13 flies which had been fed several days on a surra monkey, were emulsified in salt solution and then inoculated into 2 guinea pigs, 97 and 98. This also gave a negative result.

The experiments of the next series, in which attempts were made to transmit the surra trypanosomes through the larvæ, are obviously grossly mechanical, although the principle involved in hereditary transmission as set forth by Calkins(58) is readily recognized as also mechanical in the sense of inheritance by contact.

In this experiment the eggs produced by several hundreds of wild flies were placed, April 27, 1912, in a jar containing a medium of horse manure with fresh blood from a surra horse. The blood was lightly infected on this day. On May 2, the larvæ were changed to a clean jar with fresh, boiled manure, cooled and sprinkled generously with heavily infected blood of a monkey recently dead from surra. On May 4, pupæ had formed, emergence taking place five days later when 11 flies appeared. Forty-eight flies were fed daily as they emerged from May 9 to 13, inclusive, on monkey 2M. On May 14, 12 flies remained, and of these 7 fed on the monkey. After feeding, the flies were emulsified and inoculated into guinea pigs 93 and 94, which after the usual tests did not react.

Within two to three days after the blood was added to the fly-breeding medium, the presence of the blood diet could be detected by the terra-cotta color of the alimentary tract of the larvæ. The surra-blood-fed larvæ were not injected to demonstrate the presence of the specific organism; however, it is thought that the disease could be reproduced by animal inoculation with fresh material. Trypanosomes were found on microscopical examination in larvæ four hours after the introduction of the infected blood. These had been passed through four changes of salt solution prior to macerating for examination.

The data embodied in Table XVII represent a summary of all of the foregoing experiments in the attempts to convey the infection of surra by the agency of *Stomoxys calcitrans*.

TABLE XVII.—*Summarized data of feeding experiments with Stomoxys.*

Time after feeding on infected animals that the flies were applied.	Number of experiments.	Total number of flies used.	Number and kind of animals exposed to infected flies.	Nature of experiment, or method of applying flies.	Results.
Immediate to 48 hours.	1	25,666	3 horses	Direct completed feeding.	Negative.
5 minutes to 53 hours.	3	225	3 guinea pigs	do	Do.
10 minutes to 8 days.	25	565	6 monkeys and 19 guinea pigs.	do	Do.
21 hours to 31 days.	1	1	31 guinea pigs	do	Do.
20 seconds to 2 minutes.	11	173	11 guinea pigs	Direct interrupted feeding.	Do.
1 to 3 minutes	14	139	14 monkeys	do	Do.
25 to 30 seconds	2	63	2 horses	do	Do.
5 seconds to 1 minute.	3	274	3 guinea pigs	Successive interrupted feeding.	1 positive, guinea pig received 206 bites during 6 days.

TABLE XVII.—*Summarized data of feeding experiments with Stomoxys*—Continued.

Time after feeding on infected animals that the flies were applied.	Number of experiments.	Total number of flies used.	Number and kind of animals exposed to infected flies.	Nature of experiment, or method of applying flies.	Results.
6 hours to 67 days.	1	14	61 guinea pigs	Successive daily feeding.	Negative.
1 day to 28 days	1	190	18 monkeys	Attempts to transmit through a cyclical development.	Do.
1 day to 30 days	1	60	8 guinea pigs and 18 monkeys.	do	Do.
1 day to 42 days	1	90	18 monkeys	do	Do.
Immediate to 68 days.	11	23	12 guinea pigs	Inoculations of infected flies.	2 positive, 1 to 6 hours, 1 immediate injection.
30 seconds to 5 minutes.	6	24	6 guinea pigs	Inoculations of soiled proboscides.	3 positive, all in 30-second intervals.
Immediate	4	48	4 guinea pigs	Rubbing infected blood into fly-bitten skin.	Negative.
30 days to 38 days	1	25	1 guinea pig	Hereditary transmission, progeny of infected flies.	Do.
About 25 days	1	75	1 guinea pig	do	Do.

METHODS EMPLOYED IN FEEDING AND KEEPING FLIES FOR LABORATORY PURPOSES.

The technique employed in maintaining the normal longevity of *Stomoxys* applies equally to bloodsucking flies of other genera, for example, species of *Lyperosia* and of Hippoboscidæ. The greatest difficulty was encountered in attempting to keep flies, in either small or great numbers, in a common enclosure.

Screened stable.—In a screened stable, aside from the artificial obstacle of confinement, the difficulties presented are summed up in the presence of natural enemies, and, do what one may, it is well-nigh impossible to wholly eradicate these. Particular reference is made to the common insectivorous lizard and the ubiquitous spider. Spraying with pure cresol was effective against the individuals present, but the disinfectant did not prevent the entrance of other lizards and spiders.

Glass vessels.—Large bottles and museum jars of 3 liters' capacity were used when it was desired to confine and to feed at one time large numbers of flies. Thirty days was the longest time flies were kept alive in these containers. In this instance,

it was found necessary for the prolongation of life during the last ten days to transfer the flies to individual test tubes after each feeding. In this method with the use of glass vessels untimely death resulted from mite infestation, cannibalism, and excess of moisture.

Mite infestation.—An unknown mite, not restricted to these flies, was found both in the hypopial stage and in the adult form. The first of these stages did not prove a menace unless present in great numbers either on the body, thus precluding proper functioning of the spiracles, or on the proboscis, which prevented insertion of the beak in feeding. When the mite was present as a true parasite in the adult form, an occasional one or two did not affect the host, but when present in larger numbers they were sufficient to enfeeble the fly on account, no doubt, of lowered resistance brought about by the artificial environment.

Cannibalism.—Newstead (57) calls attention to a case of cannibalism in *Stomoxys calcitrans*. I have found it prevalent to an unusual degree. Often the disability of an individual fly attracted the attention of another more active member which promptly attempted, and usually succeeded, in puncturing the helpless fly's abdomen. This disability resulted from engorgement, infirmities resulting from broken labium, or from the wings adhering to the glass, due to an excess of moisture. Numerous cases of flies have been found actually fracturing the labium in attempting to penetrate the host's epidermis. This may result also from the fly pricking at the glass in attempting to sip moisture from the container. Such a condition, of course, makes feeding impossible, as the proboscis is not rigid enough to puncture the skin; and, as a result, the fly dies.

Excess of moisture.—Where a large number of flies are quartered together, it is difficult to prevent an excess of moisture, even though a bibulous filter paper is provided. The condition is the result of, first, excretion; and, second, probably, condensation of the moisture of the air in the bottle, when at the temperature of 20° to 26° C.

The use of individual glass tubes.—By the use of individual glass tubes the difficult problem of keeping the flies alive in captivity was most successfully solved; for the flies can be observed at all times and longevity is increased to approach the normal. Ninety-four days was found to be the maximum life of adult *Stomoxys* kept individually under laboratory conditions in glass tubes.

A test tube of 24-millimeter bore, plugged with cotton, was

found the most convenient. A piece of white filter paper conforming to the size of the tube was found ideal to regulate the moisture requirements, and this was changed at least every two or three days. It was found advantageous to change the tube not oftener than twice each week. In feeding it was not found necessary to screen the mouth of the tube. The base of the tube was directed toward the window light and the filter paper removed; the tube was then inverted immediately over the animal's body. The fly after feeding was induced to release its hold on the skin of its host by gently tapping the tube, and gradually inclining it toward the light, after which the filter paper was restored and the tube closed with a cotton plug.

The flies when not feeding were kept in the dark at a temperature between 20° to 26° C.

Martini[32] kept experimental flies, *Stomoxys calcitrans*, at a temperature of 23° C. The longevity is not stated.

METHODS OF APPLYING THE FLIES TO THE HOST.

Monkeys.—In applying large numbers of flies in a bottle, the following method was pursued. First the monkey was strapped, abdomen down, to an improvised stock by means of surgical gauze or twine, securing the wrists and ankles which were bandaged previously to prevent chafing. Then the tail was closely cropped, bound to a stout wire with straps of gauze, and thrust into a narrow-necked bottle which harbored the flies to be fed. The other end of the wire was kept at a convenient distance from the mouth of the bottle to facilitate manipulation. Wiring the tail was resorted to on account of the animal switching the appendage against the glass and crushing numerous flies.

Another method was employed with flies fed individually from tubes inverted over the thigh or other convenient portions of the monkey, held in a similar position on the stock. At least two flies could be fed at once in this manner. (Plate IV.)

Guinea pigs.—When a guinea pig was subjected to fly bites in a large museum jar it was found to be of advantage to immobilize it by strapping to a frame of brass wire. (Plate II.) This was done in order that movements of the animal would not interfere with taking the fly census during feeding, and to prevent the guinea pigs from eating the flies. Cropping the hair of this host was found to assist the parasites in feeding.

In the use of a museum jar it was necessary to hold it horizontally with the bottom toward the light. Here the majority of

flies assemble when not feeding, and the light reactions of the fly are taken advantage of in withdrawing and introducing them. If desired, ether can be used to advantage in the transfer of animals. It should be applied at the screened end of the jar, lightly enough to prevent flight, but not sufficient to stupefy the insects.

Tubes containing single flies were also applied to guinea pigs strapped to a stock. The fly was usually placed on some convenient part of the body, preferably on the side of the abdomen. (Plate V.)

Horses in sheds.—Horses for experimentation were kept in a shed screened from flies, a method commonly employed by investigators. Here it is not possible to make close and accurate examinations. Despite the fact that many thousands of flies could be applied at once, they did not, in my experience, live longer than eight days, usually dying in five days even when food was constantly available.

Dutton, Todd, and Hanington[34] made attempts to keep tsetse flies alive longer by more nearly reproducing their natural habitat in their cages. In a cubical gauze cage, 18 inches along the side, containing water and growing grass, guinea pigs and rats were used. Flies were found to feed much better when animals were immobilized in cages. Smaller cages than the above were found more advantageous for purposes of closer observations.

Horses on operating table.—In this method, the obvious advantage is in obtaining accurate data in feeding operations, and timing can be done when desirable. This method supplanted the cruder one of throwing the horse to the ground and feeding flies from inverted bottles. The violent struggling of the unwilling host is not favorable for obtaining accurate results.

In all of the methods attempted with the various animals, the hair was closely cropped with scissors. To avoid abrasions a razor was never employed. If the skin were broken in this manner and a positive result obtained, obviously it might invalidate the conclusions to be drawn from the experiment. Contamination might be produced under these conditions by the pulvilli of the feet, and possibly, though remotely, by the body hairs and wings of the insects. It was found advantageous to slightly dampen the skin of the host to make the animal odor more attractive to the fly and arousing its blood-drawing instincts.

In conclusion, it must constantly be borne in mind that all of these artificial accessories in methods may possibly jeopardize valid results by increasing the opportunities for contaminative infections.

GENERAL SUMMARY.

1. Only negative results were obtained in the attempts at direct mechanical transmission of surra with flies which were induced to bite healthy animals at intervals ranging from five minutes to three days after being permitted to complete the feeding upon infected animals. Thousands of *Stomoxys calcitrans* were employed in 29 experiments involving the use of 3 horses, 6 monkeys, and 22 guinea pigs.

2. Twenty-seven experiments were performed in attempts to transmit surra by the interruped method of feeding. All attempts proved negative *where a single application of a varying number of flies was used,* as many as 38 on a horse, and a maximum of 40 on a small guinea pig. The intervals between feeding on infected and healthy animals averaged twenty-five to forty seconds in the two instances cited.

3. In 3 trials, interrupted feeding was employed in successive daily applications. In attempting to determine the minimum number of bites necessary to infect an animal, as high as 40 were followed by negative results. The only positive result obtained was produced from a succession of 206 interrupted bites in which the flies were transferred immediately from the infected to the clean animal. The flies were applied thirty-two hours during a period of six days.

4. The results of these experiments indicate that *Trypanosoma evansi* does not develop in the body of *Stomoxys calcitrans.* Ninety-four days was the longest period in which laboratory-bred flies were tested for a cyclical development, and sixty-seven days the maximum for wild flies.

5. Organisms of surra were not found in *Stomoxys calcitrans* beyond eighteen hours after feeding on an infected animal, and the limit for infection by inoculation was ascertained in these experiments to be six hours.

6. Pathogenic trypanosomes were found in the proboscis of the fly thirty seconds after feeding on infected blood. Within one minute and thirty seconds the organisms were not present in the mouth parts in a form capable of infecting by inoculation into guinea pigs.

7. The wounds made by the labium of *Stomoxys* were not found to be a suitable channel for infection. Consequently it is not likely that surra in domestic animals is produced through this avenue by external contamination; namely, fæces, mouth parts, and pulvilli of infected flies.

8. The intimate relation in the feeding habits of *Stomoxys* and of house flies has been pointed out. *Stomoxys* has been demonstrated to provide through its bites the infection of *Musca domestica* and other dung flies. These flies have been demonstrated to act as carriers, harboring the surra organisms for several hours.

9. No evidence was obtained to indicate that *Tr. evansi* is hereditarily transmitted to the offspring of *S. calcitrans*. The larva of this fly fed on surra blood does not continue to harbor the trypanosome and the fly is "clean" upon reaching maturity.

10. It is demonstrated that the individual glass-tube method is the most suitable for applying flies in feeding on experimental animals and for keeping flies for long periods under laboratory conditions.

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ILLUSTRATIONS

(Photographs by W. H. Boyn

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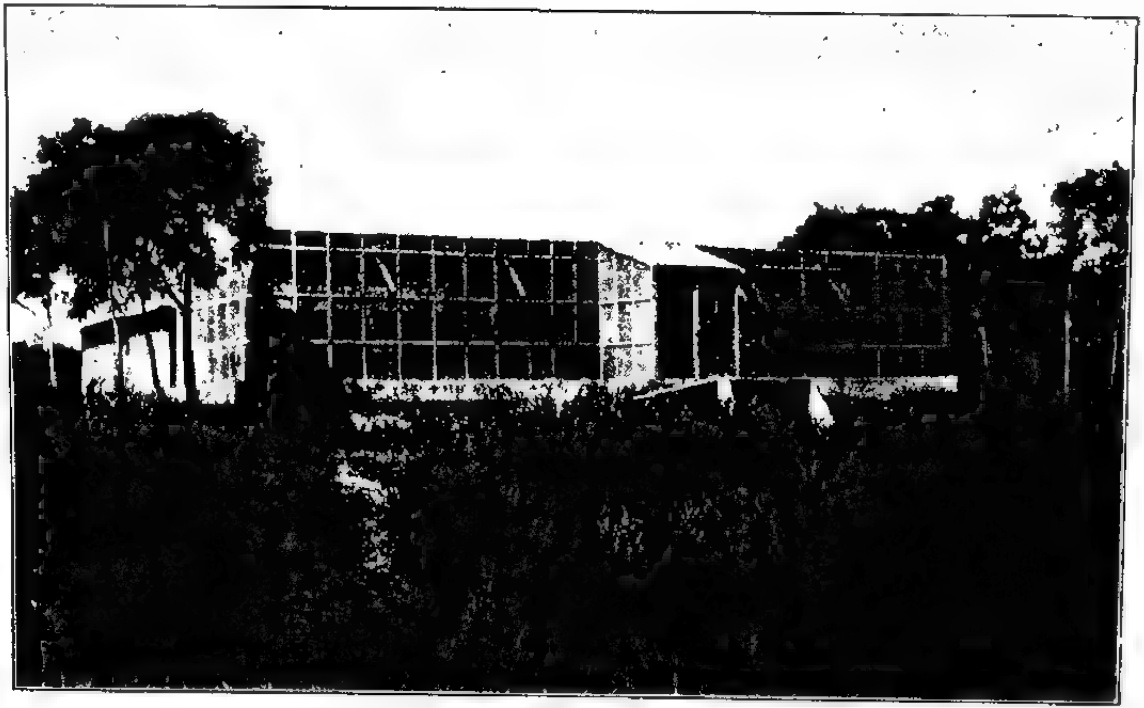


PLATE I. SCREENED STABLE FOR FLY EXPERIMENTS WITH HORSES—THE OUTER DOOR IS OPEN TO SHOW THE INTERIOR OF THE FLY-TRAP VESTIBULE.

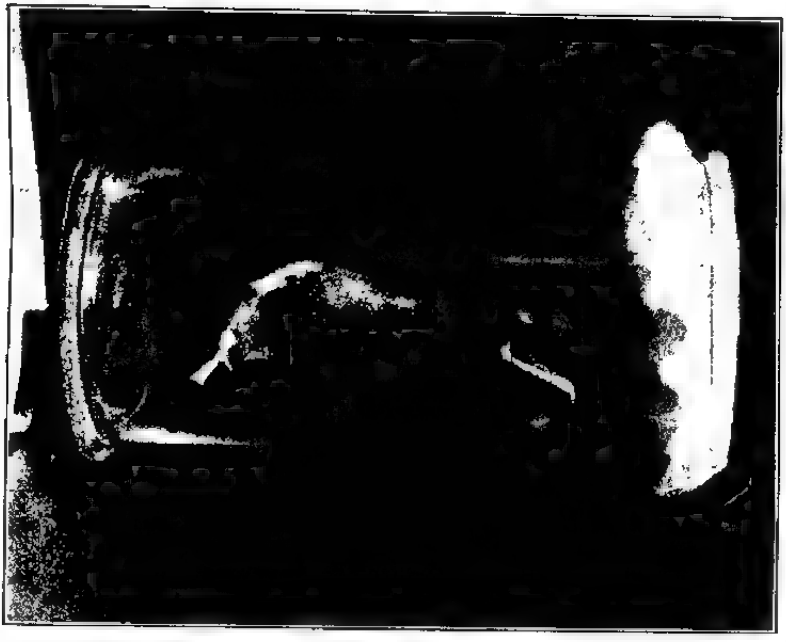
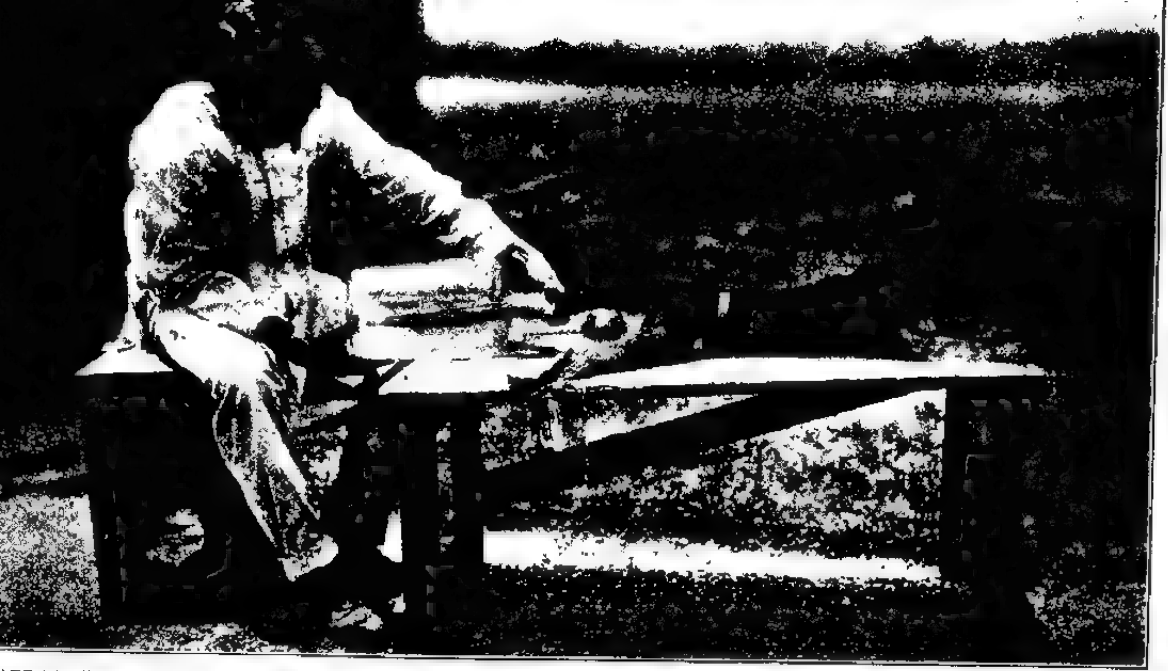


PLATE II. IMMOBILIZED GUINEA PIG IN A LARGE MUSEUM JAR FOR EXPERIMENTS WITH GREAT NUMBERS OF FLIES.



ATE III. ILLUSTRATING METHOD OF APPLYING AN UNLIMITED NUMBER OF STOMOXYS FLIES TO THE MONKEY'S TAIL INCLOSED IN A LARGE BOTTLE.

PLATE IV. ILLUSTRATING THE FEEDING OF STOMOXYS FLIES IN INVERTED TUBES ON IMMOBILE MONKEY.

PLATE V. SHOWING SINGLE TUBE APPLICATION OF STOMOXYS ON A GUINEA PIG.

THE SUSCEPTIBILITY OF COCKROACHES TO PLAGUE BACILLI INOCULATED INTO THE BODY CAVITY.

By M. A. Barber.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

The methods and results of these experiments may be illustrated by a detailed description of two or three series.

In one series 26 cockroaches, 11 *Periplaneta americana* Linn. and 15 *Rhyparobia maderæ* Fabr.,[1] all adults except 2 well-grown nymphs, were inoculated with a virulent strain of plague from a 24-hour culture. Inoculations were made under a magnifying lens with a very fine pipette of hard glass, the outer diameter of the point of which was 0.08 millimeter. The dose, approximately 0.3 cubic millimeter of a thick suspension in salt solution, was estimated in two ways. The cubic contents of the pipette below the dose mark measured with the eyepiece micrometer gave 0.4 cubic millimeter. The dose was delivered on the counting chamber of a Thoma Zeiss counter, the cover adjusted, and the cylindrical drop measured, giving 0.3 cubic millimeter. This quantity, corresponding approximately to the cubic capacity of the pipette, may be taken as approximately the dose delivered. The number of bacilli, estimated by counting dilutions, was approximately two and one-half millions, a quantity far in excess of the fatal dose for guinea pigs,[2] and enormous for insects of an average weight of about 1.2 grams.

Inoculations were made in the leg, in most instances in the basal sclerite of the dorsal surface of the coxa where the chitin is thin enough to permit the easy passage of the pipette. In order to prevent contamination with other bacteria, the surface inoculated was rubbed with a small pledget of cotton moistened with alcohol just before inoculation, and after inoculation again rubbed with the alcohol and the minute wound covered with sterile vaseline. Each insect was put after inoculation into a separate receptacle and left at a room temperature of 25° to 31°.

[1] Identifications by Charles S. Banks, Bureau of Science.

[2] *This Journal, Sec. B* (1912), 7, 251–254.

Of the 26 inoculated in this manner, 6 were dead the next day. A femur of each of them was washed with alcohol, cut off with hot scissors, and the contents pressed out at the cut surface without allowing them to touch anything not sterile. From these contents, hanging drops, smears, and cultures were made. The hanging drops were examined at once and again after seven to twenty-four hours' growth. Of the 6, two showed apparently a pure culture of plague in hanging drops examined immediately and in the smear, but all cultures showed in addition to plague-like bacilli numerous small, actively motile bacilli.

On the second day 5 more were dead. Four of these showed apparently a pure culture of plague in hanging drops and smears, and the hanging drops after growth showed apparently typical plague chains. The fifth showed a mixture of plague-like bacilli with the small motile bacilli mentioned above.

On the third day 2 more were dead. One in culture showed the small motile bacillus, the other *Bacillus prodigiosus*, both apparently in pure culture. One died on the eighth day with no indications of plague at necropsy, and 2 died on the thirteenth and fourteenth days respectively. Therefore, 12 of the 26 survived twelve days or longer—all of them *Rhyparobia maderæ*—and 10 survived two weeks or longer.

Agar-cultures were made from the four dying on the second day which showed a pure culture of apparently typical plague, and guinea pigs were inoculated subcutaneously with about one-fifth of a 24-hour agar slant. As a control, a guinea pig was inoculated with a much smaller dose of the original plague culture with which the cockroaches had been inoculated. The control died in nine days with typical lesions of plague; while of the four others only one showed signs of infection. This one died in four days with lesions in all respects typical of bubonic plague.

The nonmotile plague-like bacillus which failed to infect the guinea pigs in the three cases has not been identified. It is very improbable that it could be attenuated plague, since very large doses failed to infect guinea pigs, and since cultures on agar and in vaseline-covered broth were not typical of plague.

Of the entire 26, then, only one died of unmixed plague infection. In the case of the 10 others which died on or before the second day, plague may have contributed to their death, but in no case did plague bacilli occur in pure culture at necropsy.

In all series the commonest contaminating organism found alone or mixed with plague in insects after death was a small actively motile bacillus, very closely resembling plague in stained

preparations. Culturally its characteristics resemble those of the *Bacillus enteriditis* group. It has occasionally appeared in mammals inoculated with plague in this laboratory, and may persist with plague through several animal passages. It is pathogenic for cockroaches; for in one series of 12, inoculated with material from the lung of a monkey containing this organism probably mixed with plague, all 12 died in one day. Material from one of these insects carried to a new series gave a small proportion of fatal infections in very small doses, and a larger proportion with massive doses. A series of 5 inoculated with a pure culture of this organism isolated from a cockroach gave 3 fatal infections.

In the disappearance of plague from noninfected insects, phagocytosis must play some part. In one insect of another series, body fluid both from the leg inoculated and from a leg on the opposite side of the body was removed two hours after inoculation. Plague bacilli were found in both samples in phagocytes (in one phagocyte 18 bacilli) and outside of them.

In the series of the 26th mentioned above, the plague culture was of highly virulent strain, but was the third remove from an infected guinea pig and had been kept some days at refrigerator temperature after the first transfer from the pig. In another series, 5 large cockroaches were inoculated with material from the second transfer on agar from an infected guinea pig. Two days after inoculation 2 were found dead with numerous, apparently plague, bacilli in the body fluid. Material taken from the leg of one of the survivors showed no bacteria microscopically or culturally. All of the 3 survivors were alive and apparently well after eleven days, and 2 of them after eighteen days.

Bacilli taken from one of the infected insects of this series were grown in serum broth and this culture inoculated into 4 cockroaches. Of these four, 1 was sacrificed after two days, but showed no infection. Of the three, 2 were alive and well fourteen days after inoculation and 1 twenty-six days.

In another series, 7 were inoculated with an emulsion of the spleen of an infected guinea pig. One died in two days with apparently a pure culture of plague in the body fluid. Of the remaining six, 4 were alive after fourteen days, and 2 after twenty-seven days.

In all, 61 cockroaches were inoculated with virulent plague. Of this number, only 9 showed at necropsy a pure culture of bacilli morphologically resembling plague. Four of these cultures were inoculated into guinea pigs and only one brought about a plague infection. So of the entire 61, only 6 at most could have died

of unmixed plague infection, and in only one of these was the culture identified by guinea-pig infections.

Of the noninfected insects, at least one-half were living from twelve to twenty-seven days after inoculation. Since all which died of plague, either alone or mixed with some other bacteria, died within two days after inoculation, it seems probable that those surviving six days were not infected. At least 28 survived two weeks or more after inoculation.

In summary, it has been clearly shown that cockroaches may be infected by large doses of virulent plague bacilli; but from the fact that massive doses failed to infect a large proportion of cases, it may be concluded that these insects, especially *Rhyparobia maderæ*, are little susceptible to plague inoculated into the body cavity.

NOTES ON THE MUSCULAR CHANGES BROUGHT ABOUT BY INTERMUSCULAR INJECTION OF CALVES WITH THE VIRUS OF CONTAGIOUS PLEUROPNEUMONIA.[1]

By WILLIAM HUTCHINS BOYNTON.

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In looking up the literature concerning the muscular changes in pleuropneumonia, there has come to notice but one article on the subject by Meyer,(1) who gives a very good description of the changes. The writer agrees with him in practically every respect, but will try to bring out some points of comparison between the muscle and lung lesions which were not included within the scope of his studies.

The animals used in the experiment were young native calves, apparently in vigorous health before they were inoculated, which had the best of care during the entire experiment.

The muscle tissues used for histological purposes were taken from three calves, which died as a result of intermuscular injection of lymph secured from the thoracic cavity of animals dead of contagious pleuropneumonia.

The muscle tissue was fixed in formalin and, in some cases, in Zenker's fluid. Both paraffin and frozen sections were made, and stained with hæmotoxylin-eosin, Giemsa, Wright's stain, Jenner's stain, and Weigert's special method for fibrin.

Upon microscopic examination of the subcutaneous connective tissue, the most striking change which one notices is its marked distention, the tissue being infiltrated with a coagulated fibrinous exudate. The connective-tissue fibers are either pushed to one side or have undergone necrosis as a result of the coagulation of exudate around them.

Scattered through the distended connective-tissue spaces will be noticed isolated or confluent dark-staining areas, which vary

[1] Reprinted from Bull. No. 20, Bureau of Agriculture of the Government of the Philippine Islands.—The Bureau of Agriculture is indebted to the biological laboratory, Bureau of Science, for the use of the laboratory facilities utilized in carrying out this work.

[2] Archibald R. Ward, chief.

approximately from 50 to 160 microns in diameter. Located in or near the center of these areas are either single or ramified blood vessels distended with blood. In some instances these contain a considerable number of leucocytes, which are as a rule situated near the vessel wall, showing that the blood stream had been retarded in its flow. It is a known fact that "a greater or less number of leucocytes pass over into the peripheral plasma zone, when the slowing of the circulation has reached a certain degree."(2)

In some instances the vessel walls apparently have not deviated from normal to any great extent, while in others migration of leucocytes and diapedesis of red cells may be observed.

The dark-staining areas situated around the blood vessels take on different appearances at different stages of development, of which four can be easily recognized.

First. The congested blood vessel is surrounded by a zone of leucocytes, being composed of both round cells and polynuclears. These areas average from 50 to 70 microns in diameter.

Secondly. The congested blood vessels are surrounded by a zone of round cells and polynuclear leucocytes. Around this is a zone of broken down leucocytes and cell detritus intermixed with the fibrinous exudate. These areas average from 80 to 120 microns in diameter.

Thirdly. The congested blood vessels are surrounded by a light-staining zone of new-forming connective-tissue cells, intermixed with a few leucocytes, and in some instances new-forming blood vessels which are congested. Around this zone of new-forming connective tissue is a deeper staining zone composed of leucocytes, and situated around this area is a zone of broken down leucocytes and cell detritus extending into the fibrinous exudate. These areas average from 100 to 160 microns in diameter. (Plate IV.) Hence it will be noticed that as the irritant persists, a chronic inflammation is produced, which leads to the production of new-forming connective tissue and of blood vessels.

Fourthly. In a few instances blood vessels are completely occluded by thrombus formations. In these cases the vessel walls are degenerated to a considerable extent and leucocytes situated around them are undergoing karyorrhexis.

Where the blood vessels are situated close together, the inflammatory zones around them coalesce, forming oblong dark-staining areas or bands, with the congested vessels in the center, and the deeper staining areas of leucocytes along the periphery.

Summarizing the changes which take place in the older lesions,

there will be noticed proceeding from the inside outward: First, the congested blood vessels; secondly, a zone of new-forming connective tissue, in some instances containing new-forming blood vessels; thirdly, a zone of leucocytes; fourthly, a zone of broken down leucocytes and cells detritus; fifthly, the coagulated fibrinous exudate. All of these changes are shown more or less distinctly in Plate IV.

The vascular changes in the subcutaneous connective tissue are very similar to those occurring in lung tissue affected with contagious pleuropneumonia. The writer has noticed new formation of connective tissue around the blood vessels in the lung, especially in the vicinity of sequestra where the area involved is being walled in by a fibrous capsule. The earlier vascular changes of the subcutaneous tissue also simulate the changes seen around the arteries and veins in affected lung tissue, also the thrombi agree with those found in the veins of affected lung tissue.

The changes in the epimysium are very similar to those observed in the subcutaneous connective tissue. The bands of connective tissue surrounding the muscle bundles are markedly distended with a fibrinous exudate, causing degeneration of the connective-tissue fibers or pushing them to one side. The vascular changes, also, correspond to those already described in the subcutaneous tissue.

One very striking lesion is the accumulation of leucocytes, in various stages of degeneration, into foci and lines along the margin of the epimysium. These take a deep stain and can thus be traced with the unaided eye, forming very distinct lines which mark off the borders of the epimysium as it extends through the muscle tissue. This border of cells extends along the edges nearest the muscle tissue, and even extends around the individual muscle fibers, causing them to degenerate. The changes in question are brought out distinctly in Plates I (*a*) and II (*b*). These borders of cells correspond exactly with those found along the edges of the interstitial tissue in lungs affected with contagious pleuropneumonia, and are regarded by Smith(3) to be one of the characteristic lesions of that disease.

From the inner surface of the epimysium, septa are sent in which divide the muscle into a number of large secondary bundles. These septa are markedly distended with a fibrinous exudate which may contain leucocytes scattered throughout. As a rule, the leucocytes are thickest along the edges of the septa nearest the muscle, simulating the line formation described above.

One of the striking lesions in these septa is the enormous distention of the blood vessels, as shown in Plate I (*e* and *f*). In some instances, these vessels contain large numbers of leucocytes situated around the periphery; in others, they are scattered uniformly throughout the blood stream, indicating that there has been a slowing or even complete stasis of the flow. This is undoubtedly caused by thrombus formation, as both parietal and obturating thrombi are present, Plate I (*b* and *e*). The presence of a parietal thrombus partly occluding the vessel is shown in Plate I (*e*). This particular thrombus is of the gray type, being composed of fibrin and leucocytes, while the rest of the vessel is filled with blood. The vessel wall is undergoing degeneration. A few mixed and two organizing thrombi have been noticed, but as a rule they are of the gray variety.

The accumulations of leucocytes around the blood vessels in the septa are not so marked as those seen in the epimysium and subcutaneous tissue.

Extending from the septa are connective-tissue bands designated as the perimysium which divide the muscle into primary bundles or fasciculi. This perimysium is also distended with a fibrinous exudate intermixed with leucocytes. In places in the perimysium thus affected, the vessels are distended with blood, both parietal and obturating thrombi being present. Plate I (*b*) shows an obturating thrombus becoming organized. In many instances the vessel walls are undergoing degeneration, emigrating leucocytes are seen passing through them, and also diapedesis of red cells occurring.

The perimysium is not all affected alike. For instance, in Plate I (*h*) it is not so distended, contains a few leucocytes, some fibrin, and also new-forming connective-tissue cells, thus taking on more of the chronic type of inflammation. This may be accounted for by the fact that the pleuropneumonia virus seems to attack primarily the connective tissue, and as the process extends downward into the areas where there is less connective tissue there would naturally be fewer changes as there is less material for the virus to work upon. Where the virus is not sufficiently abundant to bring about marked changes, its continuous irritating action may be the cause of the chronic inflammatory process. In those areas where new-forming connective tissue is present, new-forming blood vessels are occasionally seen, slightly congested, but no thrombus formations have been noticed in these particular parts.

The perimysium sends off connective-tissue fibers which pass between the individual muscle fibers. These constitute the endomysium. Plates II (*d*) and III (*d*) show the endomysium around the individual muscle fibers. In places the endomysium is infiltrated with a slight fibrinous exudate intermingled with leucocytes, while in other places it has become hyperplastic by the new formation of connective tissue. Now and then new-forming blood vessels are found in this hyperplastic endomysium, Plate III (*b*), showing the presence of a productive inflammation.

It will be noticed that the muscle tissue situated closest to the epimysium has undergone the most marked pathological changes, Plates II (*e* and *f*) and III (*c* and *e*). This seems plausible as the predominant changes are situated in the epimysium, the exudate of which by continuity extends among the muscle fibers. In these areas the endomysium is distended with a fibrinous exudate which is composed of large numbers of leucocytes, especially in the vicinity of the bands of cells mentioned above. Under the influence of this exudate, the muscle fibers become in some instances filled with vacuoles, lose their striations, take on a granular appearance, and lose their nuclei. Thus they present a typical picture of granular degeneration which may be brought about by the coagulation of the exudate around them, shutting off their nutrition, or the toxin from the pleuropneumonia virus may have a vital influence.

Another characteristic lesion is the degeneration atrophy of the muscle fibers. In many cases the fibers have completely disappeared, leaving the endomysium surrounding the spaces which were once occupied by them.

Upon examination of sections of tissue thus affected, muscle fibers are found in all stages of degeneration, and as the degeneration advances the fibers become more shrunken and finally disappear entirely.

CONCLUSIONS.

1. From all appearances, the contagious pleuropneumonia virus seems to have a specific action upon muscle and connective tissue, affecting chiefly the connective-tissue elements.

2. The appearances suggest that the virus multiplies in the lymph spaces of the connective tissue and blood vessels, gradually working its way through the walls of the blood vessels, causing an inflammation of the intima and thus giving rise to thrombus formations.

3. The virus having invaded the tissue gives rise to a serofibrinous exudate, intermingled with groups of leucocytes, leading to thrombosis of both lymph and blood vessels.

4. The muscle lesions correspond with the lung lesions of contagious pleuropneumonia in the following respect:

(*a*) Thrombus formation occurs in the veins in both tissues.

(*b*) The inflammatory areas around the blood vessels are similar.

(*c*) The connective tissue is chiefly affected in both tissues.

(*d*) The abundant serofibrinous exudate is present in both.

(*e*) The deep-staining line of leucocytes along the edge of the connective tissue is characteristic in both tissues.

(*f*) The tendency toward a chronic productive inflammation is present in both.

5. Thus in summing up all the lesions, the lung and muscle lesions are found to correspond in practically every respect.

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ILLUSTRATIONS.

(From photomicrographs in the collection of the Bureau of Science, Manila, P. I.)

PLATE I.

a Markedly distended epimysium, showing the wall of infiltrating leucocytes along its border.
a' The fibrinous exudate containing very few cells in the central part of the distended epimysium.
b An obturating white thrombus occluding a vein in the perimysium.
c New-forming connective tissue causing a hyperplasia of the perimysium.
d A partly occluded artery in the septa.
e A mixed parietal thrombus in an enormously engorged vein of the septum.
f An enormously congested vein of the septum, showing the numerous leucocytes in the blood; also their migration through the degenerated wall of the vessel.
g The atrophied and degenerating muscle fibers throughout the specimen.
h Hyperplastic perimysium containing a few leucocytes but mostly new-forming connective tissue.

PLATE II.

a Fibrinous exudate containing very few cells, located in the central part of the markedly distended epimysium.
b The well of infiltrating leucocytes along the edge of the markedly distended epimysium.
c Vein containing blood with numerous leucocytes. In the walls of the vessel are migrating leucocytes.
c' New-forming connective-tissue cells with some migrating leucocytes.
d Hyperplastic endomysium composed mostly of new-forming connective tissue, with a few migrating leucocytes.
e Vacuoles left where the muscle fibers have completely disappeared.
f Atrophied muscle fiber, showing space it should occupy, and the hyperplastic endomysium around it.

PLATE III.

a Wall of leucocytes along the edge of the epimysium.
b New-forming blood vessel in the hyperplastic endomysium.
c One of the many vacuoles where the muscle fibers have entirely disappeared.
d New-forming connective tissue with a few migrating leucocytes forming the hyperplastic endomysium.
e Two of the numerous atrophied and degenerating muscle fibers.

PLATE IV.

a Congested blood vessel in the subcutaneous tissue, showing numerous leucocytes in the blood.
b Zone of new-forming connective tissue around the blood vessel.
c New-forming blood vessels distended with blood in the connective-tissue zone.
d Zone of leucocytes, principally polynuclears with a few round cells.
e Zone of broken down leucocytes and cell detritus.
f Fibrin from the inflammatory exudate.

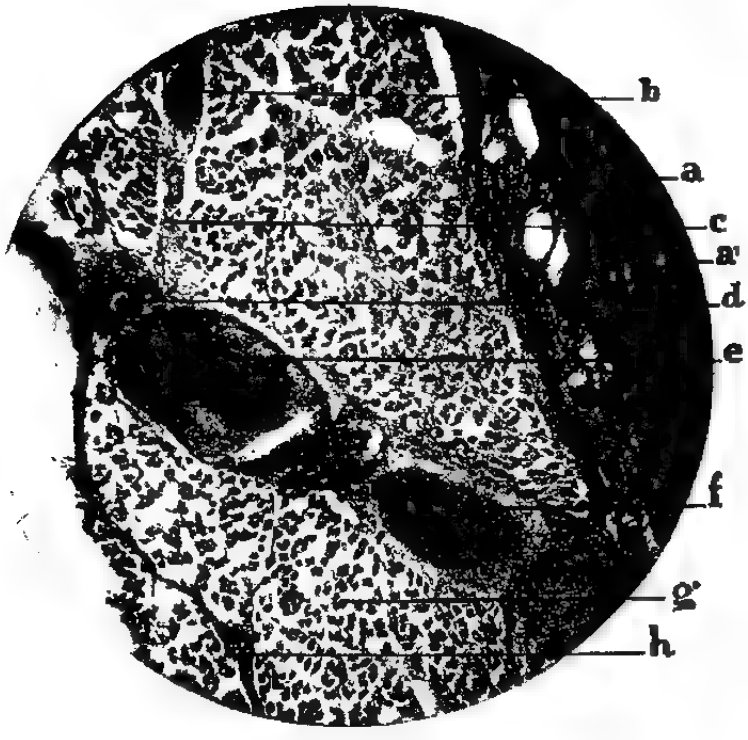

PLATE I.

PLATE II.

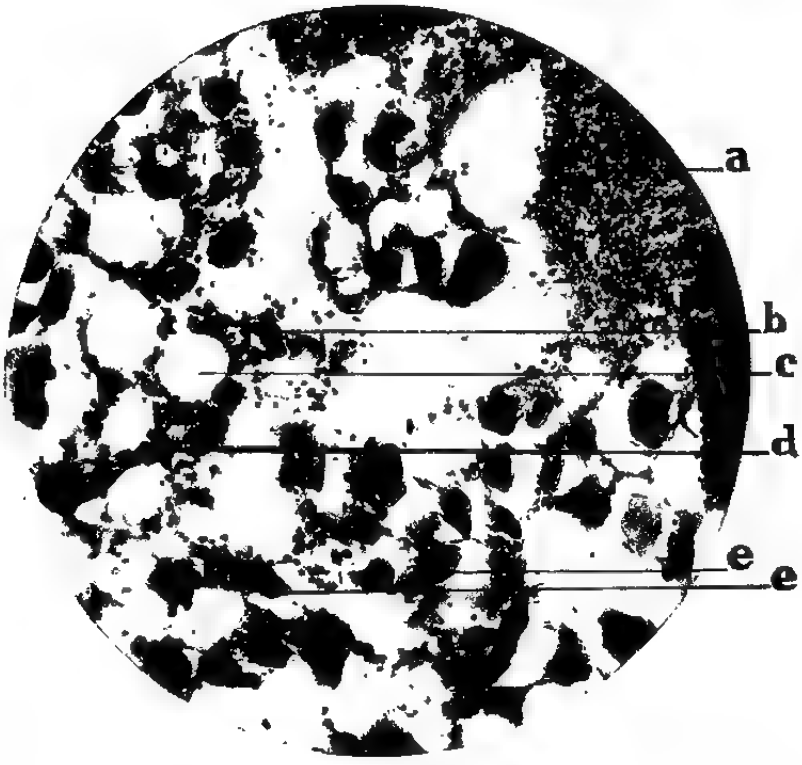

PLATE III.

PLATE IV.

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